INFLUENCE OF HYDROLOGY AND DENITRIFICATION ON NUTRIENT DYNAMICS IN COASTAL HEADWATER STREAMS

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ABSTRACT

SARA K. MCMILLAN: Influence of hydrology and denitrification on
nutrient dynamics in coastal headwater streams
(Under the direction of Hans W. Paerl and Michael F. Piehler)

Watershed-derived nitrogen (N) inputs from coastal watersheds can have a significant impact on estuarine function, with land use and stream network characteristics altering the timing, quantity and quality (labile vs. refractory) of this source. Excess N in estuarine ecosystems has led to increased rates of primary production (eutrophication), reduced biodiversity, habitat degradation and food web alterations. Nitrogen retention is particularly high in shallow headwater streams due to high biological activity, increased sediment surface to streamwater volume ratios and the fact that low order streams encompass a large proportion of total stream length within the stream network. Denitrification is one component of instream N retention that is particularly important because it removes N from the aquatic ecosystem. Two coastal plain watersheds of contrasting land uses (agricultural and silvicultural) located adjacent to the Neuse River Estuary were studied to assess the effect of hydrology on nutrient export, the factors controlling rates of denitrification in streambed sediments and the contribution of denitrification to instream N removal. Nitrate export was greatly affected by hydrodynamic conditions, with nutrient pulses observed during storm events, often increasing instream concentrations by 1-2 orders of magnitude. Factors that controlled

denitrification varied by land use. Nitrate, organic carbon and elevated temperature stimulated rates in agricultural streams, but had minimal impact in silvicultural streams.

Instream nutrient retention determined by mass balance calculations showed the stream to be a significant sink for ammonium (46%) and phosphate (14%), but not nitrate. High rates of denitrification observed in the agricultural sediments following nitrate additions showed significant potential for denitrification, which responded linearly to increasing nitrate concentrations. However, the ability of denitrification to attenuate storm pulses of nitrate depends largely on hydrological transport of nitrate-rich streamwater to denitrifying communities in streambed sediments. Management of these drainage networks, including channel modifications to increase hyporheic flow (i.e. addition of woody debris or other channel obstructions) or to increase retention time (i.e. flashboard risers or streamside wetlands) may help reduce downstream export in streams that support high rates of denitrification.

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Ar	argon
AIT	acetylene inhibition technique
B	baseflow
BA	silvicultural watershed outlet; 2nd order canal sampling site
BC	silvicultural 4th order creek sampling site
BC-M	marsh adjacent to BC (silvicultural 4th order creek sampling site)
BMPs	best management practices
C	carbon; concentration
°C	Celsius degrees
CHN	carbon, hydrogen, nitrogen
cm	centimeters
CO_2	carbon dioxide
C_s	concentration in the model segment
C_{s-1}	concentration in the adjacent upstream model segment
CS	agricultural 4th order creek sampling site
CS-M	marsh adjacent to CS (agricultural 4th order creek sampling site)
d	channel depth
DIN	dissolved inorganic nitrogen
DOC	dissolved organic carbon
DON	dissolved organic nitrogen
F	fall
FC	agricultural 2nd order canal sampling site

FD	agricultural 1st order ditch sampling site
FY	flow year
g	grams
h	hour
H_2	hydrogen gas
ha	hectares
k	loss rate
kg	kilograms
km	kilometers
L	liters
m	meters
mg	milligrams
MIMS	membrane inlet mass spectrometer
mL	milliliter
mm	millimeters
μm	micrometers
μM	micromolar
N	nitrogen
N_2	molecular nitrogen, nitrogen gas
NH_4-N	ammonium
N_2O	nitrous oxide
NO	nitric oxide
NO_3-N	nitrate

O_2	oxygen
P	phosphorus
POC	particulate organic carbon
PON	particulate organic nitrogen
PO_4-P	phosphate
Q	discharge
s	seconds
S	storm events
SE	standard error
SOD	sediment oxygen demand
Sp	spring
Su	summer
SWC	Southwest Creek; agricultural watershed outlet station
TN	total nitrogen
u	stream velocity
U	uptake rate
V_f	mass transfer velocity
W	winter
WD	silvicultural 1st order ditch sampling site
x	distance between model segments
y	year

CHAPTER 1

RATIONALE AND RESEARCH OBJECTIVES

1.1. Research rationale

Watershed-derived nitrogen (N) inputs from watersheds adjacent to coastal and estuarine waters can have a significant impact on estuarine function, with land use characteristics altering both the timing, quantity and quality (labile vs. refractory) of this source (Basnyat et al. 1999, Allan 2004). Excess N in estuarine ecosystems has led to increased rates of primary production (eutrophication), reduced biodiversity, habitat degradation and food web alterations (Nixon 1995, Rabalais et al. 2001, Paerl et al. 2002). Large-scale drivers of estuarine productivity include nonpoint and point source inputs from the watershed, riverine flow and atmospheric deposition. These forcing features influence estuarine function on large spatial and temporal scales (Mallin et al. 1993, Peierls and Paerl 1997, Paerl et al. 1998). However, internal nutrient cycling, groundwater inputs and storm-driven nutrient pulses from proximate watersheds can have significant impact on shorter time and space scales (Paerl et al. 1998).

Low-lying coastal watersheds exhibit a distinct hydrology due to the construction of drainage systems to facilitate crop production, residential development and silviculture (Amatya et al. 1996, Lebo and Herrmann 1998). These drainage systems consist of regularly aligned, straight streams with little geomorphic heterogeneity. The flat topography and low infiltration capacity causes rapid peak flows during storm events followed by very low flows during baseflow. Not only does this complex hydrology impact volumetric discharge but also has significant implications for nutrient transformations in headwater streams. Lowered hydraulic retention times during storms indirectly influence biogeochemical processes by reducing the proportion of water volume in contact with biologically active sediment communities. Additionally, hydrodynamic transport of nutrient-rich streamwater to these communities is limited in streams with little geomorphic heterogeneity (i.e. meandering reaches, riffle-pool sequences or stream obstructions).

Headwater streams have been shown to play a critical role in nutrient transformations on the landscape (Galloway et al. 2003, Seitzinger et al. 2006), but the degree to which these attributes can be transferred to coastal engineered drainage systems is uncertain. The majority of the N removal in these streams has been attributed to denitrification with loss rates as much as 45% per day (Alexander et al. 2000, Peterson et al. 2001). As NO_3-N enters the stream, it is subject to multiple processes that contribute to retention (assimilation, burial and reduction to NH_4-N) and removal (denitrification). Assimilation by macrophytes, algae and bacteria generally represents short-term storage because this organic N is either released in dissolved forms or remineralized whereas denitrification results in a loss of N from the riverine system. Direct measurement of denitrification rates in streambed sediment can be high in many cases, particularly in agricultural watersheds where concentrations of both organic matter and nitrate are high (Jansson et al. 1994, Laursen and Seitzinger 2002, Royer et al. 2004, Schaller et al. 2004, Smith et al. 2006). Understanding the impact of denitrification on nitrate removal in these coastal streams is critical for management of these systems to minimize adverse downstream impacts.

1.2 Study objectives

1.2.1 Linking hydrology and biogeochemistry

Objective: Elucidate the linkage between hydrology in modified stream networks and nutrient retention in coastal watersheds in close proximity to sensitive estuarine waters

Hypothesis: Storm-driven nutrient pulses will be greater in the agricultural watershed compared to the silvicultural watershed due to increased terrestrial N inputs. Nutrient retention in the engineered agricultural stream network will be significantly greater for NH_4-N and PO_4-P compared to NO_3-N because of the mobility of NO_3-N and the preferential utilization of NH_4-N by algae and bacteria. Additionally, nutrient retention in both watersheds will strongly depend on stream discharge, decreasing as hydraulic retention time increases.

1.2.2 Spatial and temporal variability of denitrification

Objective: Characterize spatial and temporal patterns of denitrification in headwater stream sediments and quantify the potential for denitrification to remove nitrate from the stream network.

Hypothesis: Denitrification rates will vary on a seasonal basis in response to temperature fluctuations. Additionally rates will be greatest in first order streams and decrease as stream order increases. As such the potential for denitrification to remove watershed N will be greatest in the low order streams.

1.2.3 Variables affecting denitrification

Objective: Identify the factors controlling denitrification rates in headwater streams in two contrasting land uses: agricultural and silvicultural.

Hypothesis: Denitrification rates will greater in the agricultural stream network as a result of elevated instream nitrate and more biologically available organic matter in the form of algal biomass. Nitrate will be the dominant controlling factor in both land uses with carbon and temperature influencing rates secondarily.

1.2.4 Impact of denitrification on nitrate export from the agricultural watershed

Objective: Quantify the contribution of nitrate removed in agricultural streambed sediments via denitrification as a proportion of the total instream removal determined from a mass balance analysis.

Hypothesis: Elevated rates in the agricultural stream due to high nitrate concentration will remove a significant proportion of nitrate. The proportion removed will be greater during baseflow compared to storm events because total loads are lower and retention times are greater.

1.3 Denitrification in aquatic ecosystems

Denitrification is carried out by facultative heterotrophic bacteria during the decomposition of organic matter. These bacteria use oxygen as an electron acceptor in aerobic environments, but switch to NO_3-N in anaerobic environments. These bacteria are biochemically and taxonomically very diverse. Most are heterotrophs and some utilize one-carbon compounds, whereas others grow autotrophically on hydrogen (H_2) and carbon dioxide (CO_2) or reduced sulfur compounds (Knowles 1982). The major end product is gaseous nitrogen (N_2) with lesser production of nitrous oxide (N_2O) and nitric oxide (NO). All end products are released to the atmosphere, constituting a removal of N from the system. In systems with excess N, denitrification is a desirable process because it limits transport to downstream waters where large inputs of N can lead to excessive productivity of aquatic plants and algae.

Additional physical and biological processes influence N dynamics in stream ecosystems, including uptake by plants, algae and bacteria, physical adsorption and sedimentation of particulate N. While assimilative uptake can have significant impacts on instream attenuation (Hamilton et al. 2001, Mulholland 2004), organic N in plant and algal biomass will eventually be remineralized and returned to the system. In fact, many streams function as sinks of dissolved inorganic nitrogen (DIN) and sources of dissolved organic nitrogen (DON) (Cooper and Cooke 1984).

Quality of the organic matter in aquatic ecosystems controls the balance between N mineralization or ammonification (the release of NH_4-N from decomposed organic matter) and incorporation of NH_4-N into bacterial biomass (Schlesinger 1997). At high C:N ratios, N is sequestered in biomass, whereas at lower ratios, NH_4-N is released. Remineralized NH_4-N can be utilized by nitrifying bacteria and converted to NO_3-N which can then be converted to N_2 gas by denitrifying bacteria. Nitrification proceeds solely under aerobic conditions while denitrification requires anaerobic conditions. As such, denitrification zones are often found at the interfaces of oxic/anoxic zones where the supply of labile organic matter and NO_3-N co-occur. Coupled nitrification-denitrification in these interfaces has been demonstrated in many aquatic environments including stream, lake and estuarine sediments.

Nitrogen cycling in aquatic ecosystems is complex and a variety of approaches have been used to understand the mechanisms governing N retention. The nutrient spiralling concept describes the downstream movement of N as it cycles between organic N in biomass and DIN in the water column (Newbold et al. 1981). Numerous reach scale assessments have used this approach to assess instream retention across multiple biomes (Webster et al. 2003, Mulholland et al. 2004). This approach provides valuable information regarding the net retentive capacity of a stream but does not identify specific biological and physical processes that control this retention. Mass balances approaches have been used to quantify net removal of N along a stream network. However, differences between inputs and outputs are often attributed to denitrification without measuring the process directly (Alexander et al. 2000, Seitzinger et al. 2002).

Measurement of denitrification rates directly simplifies inter-site comparisons by removing confounding environmental variables, particularly hydrological controls (groundwater inputs, precipitation patterns and hyporheic flow). Laboratory measurements also allow experimental manipulations to be conducted to isolate potential controlling factors. Several methods for measuring denitrification exist and all have limitations and benefits. Denitrification rates were measured in this study using membrane inlet mass spectrometry (MIMS) (Kana et al. 1994, Poe 2004). Since, this method does not require addition of tracers or inhibitors, denitrification can be measured in undisturbed sediment cores. However, a disadvantage of this method is that by measuring changes in the water column N_2 concentration, competing processes of denitrification and N_2 fixation can not be isolated.

Extrapolating laboratory measurements to whole ecosystems is hindered by the heterogeneity of stream bed sediments, temporal variability of substrate availability, complex interactions among different N cycling processes, and the spatial variability of water-sediment interactions. This study used a combination of both laboratory and field approaches to determine the relative importance of denitrification to instream NO_3-N removal. Nitrogen mass balance data is critical for putting these laboratory rate measurements in context of ecosystem inputs and exports.

CHAPTER 2

HYDROLOGIC CONTROLS ON SOLUTE EXPORT IN AGRICULTURAL AND SILVICULTURAL COASTAL WATERSHEDS

2.1 Introduction

Biogeochemical processes influence the concentration of nutrients during transport through stream networks, resulting in a net decrease downstream (Alexander et al. 2000, Peterson et al. 2001). It was estimated that as much as 76% of the N entering a watershed's stream network may be retained (permanently removed via denitrification or temporarily retained through biotic sequestration) within streams in the northeastern United States (Seitzinger et al. 2002). Alteration of nutrient concentrations by riverine processes during transport also changes the timing, quantity, and quality (labile vs. refractory) of nutrients exported to downstream ecosystems (Meyer and Likens 1979, Webster et al. 2003, Mulholland 2004). Understanding the processes controlling the temporal variation in nutrient delivery and availability is critical in coastal environments where nutrient enrichment can have many negative impacts including eutrophication, habitat degradation and decreased biodiversity (Nixon 1995, Boesch et al. 2001).

Land use changes in coastal areas have been linked to elevated instream nutrient concentrations and degradation of headwater streams (Basnyat et al. 1999, Allan 2004). Natural channels with complex heterogeneity and meandering reaches are being replaced by straight, homogenous drainage ditches. Increased development accompanied by changes in impervious area (due to compaction of native soils and construction of impermeable surfaces) and modification of drainage systems has lead to drastic changes in watershed hydrology. Peak storm flows are greater and baseflow is reduced due to decreased infiltration. Greater flow velocities during storm events lead to increased bed scouring and movement of channel sediments. Several unique features that distinguish coastal stream networks from those inland, including flat topography, organic rich peat soils and tidal influences from both lunar and wide driven tides, present additional challenges in understanding and predicting alterations in nutrient cycling.

This study investigated the interactions between hydrology and nutrient retention in two small coastal watersheds (silviculture and agriculture) in eastern North Carolina, USA that ultimately discharge to the Neuse River Estuary. In the agricultural watershed, additional detailed monitoring data at upstream and downstream stations along a typical 2nd order reach were used to calculate total retention of nitrate (NO_3-N), ammonium (NH_4-N) and phosphate (PO_4-P) over a 16-month period of record. A previously developed model of the stream network was revised to predict instream retention of NH_4-N and PO_4-P.

2.2 Methods

2.2.1 Site description

Agricultural and silvicultural watersheds in eastern North Carolina were monitored for instream water quality, nutrient loadings and volumetric discharge from August 2003 through July 2006 (Figure 2.1). Each watershed has a single land use and both watersheds are located in close proximity to each other. This eliminated confounding variables and facilitated the analysis of climate and precipitation impacts on nutrient dynamics based on land use type.

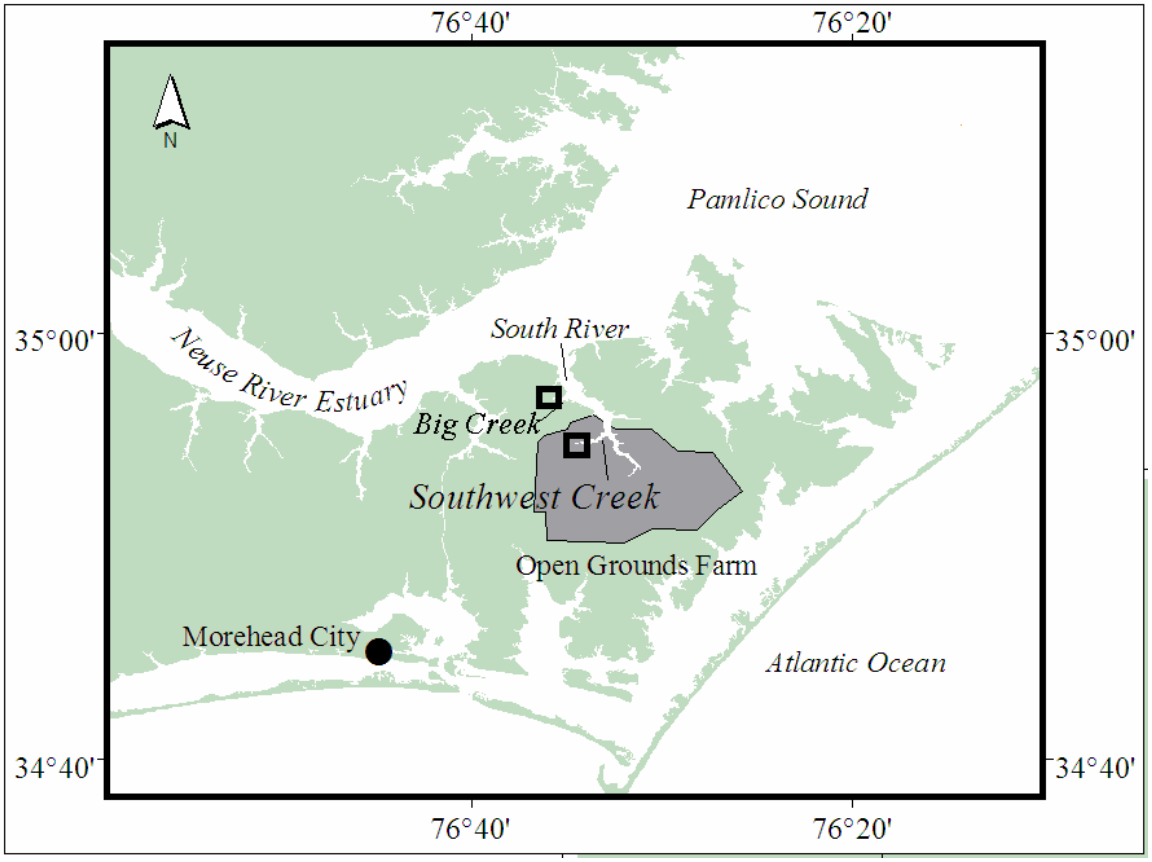

Figure 2.1. Location of the study area in eastern North Carolina, USA. The shaded area is Open Grounds Farm.

The agricultural watershed is located on Open Grounds Farm, a 182 km^2 row crop operation. The Southwest Creek sub-watershed is 7.7 km^2 and is planted with corn and soybean crops in an annual rotation. The stream drainage network consists of a regular arrangement of engineered first order ditches and second order canals that are entrenched 1 m and 3 m deep, respectively. These drain to Southwest Creek which subsequently discharges to the South River estuary. The watershed outlet (SWC) was monitored throughout the study period for discharge and water chemistry. A second monitoring station (FD) was deployed in April 2005 at the outlet of one of the first order ditches

(Figure 2.2) to measure solute export data from a 1st order ditch and allow estimation of instream nutrient retention.

The majority of the native riparian vegetation was removed to facilitate planting of crops to the edge of the stream. The lack of a riparian canopy allows high amounts of light to reach the stream surface resulting in proliferation of algae and macrophytes in the stream and along the banks. The surface sediments in 1st order ditches were organic-rich peat soils while the canal sediments were a coarse to medium grained sand covered by varying accumulations of silt and plant detritus. Canal sediments were regularly reworked during high flow events which covered or scoured submerged vegetation from the streambed. The drainage network is maintained by annual dredging activities that remove woody debris, submerged and emergent vegetation and other channel obstructions. Stream gradients were approximately 0.12%.

The silvicultural watershed is located in a forest managed by Weyerhaeuser Corporation. It is a 2.6 km^2 watershed planted with loblolly pine (*Pinus taeda*) that drains to Big Creek which in turn flows into the South River estuary. Fertilizer was applied during the spring and summer at key times during the tree's life cycle. Flow and water chemistry were monitored at the watershed outlet (BA) throughout the study period. The drainage network is comprised of a series of engineered ditches, similar to the agricultural watershed. In contrast, it has extensive riparian vegetation resulting in a shaded water surface. Because of the reduced surface irradiance and limited fertilization, algal blooms were not observed in this watershed. Minimal maintenance activities result in ditches with woody debris, leaf litter and vegetation along the stream banks.

2.2.2 Flow and nutrient load monitoring

Flow velocity and depth were monitored continuously throughout the study period (August 2003 – July 2006). Flow years (FY) were defined as the period from August through July. Automated samplers (ISCO model 6700) were placed in drainage pipes at the outlet of each watershed (BA and SWC) and at an upstream station (FD) in the agricultural watershed. Volumetric flow rates were calculated at 30 minute intervals using the cross sectional area of the pipe, flow velocity and depth. In addition, water quality parameters (temperature, dissolved oxygen, pH, and conductivity) were recorded every 30 minutes (Yellow Springs Scientific Instrument model 600R). Water samples were collected weekly from each station for analysis of NO_3-N, NH_4-N, PO_4-P, total nitrogen (TN) and dissolved organic carbon (DOC). In addition, more frequent automated sampling was conducted to enhance resolution during storm events. Rainfall data were collected at stations BA and SWC on 30 minute intervals via a collection device connected to the ISCO samplers.

Water samples were filtered through Whatman GF/F glass fiber filters (25mm diameter, 0.7 μm nominal pore size) and the filtrate was analyzed with a Lachat Quick-Chem 8000 automated ion analyzer for NO_3-N, NH_4-N and PO_4-P concentrations using standard protocols (Lachat Instruments, Milwaukee, WI, USA: NO_2/NO_3 Method 31-107-04-1-A, NH_4 Method 31-107-06-1-A and PO_4-P Method 31-115-01-3-G). Dissolved organic carbon concentrations were measured using a high temperature combustion technique on a Shimadzu model TOC-500, equipped with an ASI-5000A autosampler.

Water quality samples were collected with a fine temporal resolution during storm events facilitating the development of a continuous record of nutrient concentrations by interpolating between observed concentrations during each event. Total nutrient loads exported during isolated storms in the study period which were unable to be monitored were estimated by using mass to volume ratios of similar storms (similarities included intensity, duration and antecedent precipitation). A graphical baseflow separation method that accounted for watershed size was applied to identify the baseflow component of total stream flow during storm events (Dingman 1994). To quantify the nutrient load attributed to groundwater inflow during storm events, the immediately preceding dry weather baseflow concentration was applied to the proportion of flow attributed to baseflow. A mass balance approach was then used to calculate the resulting storm flow contribution for each event. Baseflow nutrient loads were calculated using concentrations from weekly sampling events. Two hurricanes made landfall during this monitoring period, Hurricane Isabel during September 2003 and Hurricane Ophelia during September 2005. These storms necessitated the evacuation of instrumentation from the field site due to high flow conditions that persisted for several weeks and prevented immediate redeployment of instruments.

2.2.3 Event-averaged concentrations

To compare seasonal and interannual variability in solute export during storms, event-averaged concentrations were estimated for NO_3-N, NH_4-N, PO_4-P and DOC. The total mass discharged during each rainfall event was obtained by integrating the product of the 30-minute flow discharge and the solute concentrations over the event duration. Volume-integrated concentrations were determined by dividing the total mass by the total volumetric discharge measured over the same storm duration. These concentrations were calculated for all events in which detailed water chemistry was available.

Each storm was characterized based on antecedent conditions prior to each storm. Dry antecedent conditions were defined as no rainfall events occurring 10 days prior to the storm. Wet antecedent conditions were defined as a rainfall event occurring less than 4 days prior to the storm event. Two dry periods (9/2004 – 4/2005 and 11/2005 – 7/2006) and one wet period (5/2005 – 10/2005) were well defined during the study period. Storm characteristics were compared during these times to assess the importance of antecedent conditions on solute export.

2.2.4 Nutrient and flow balance

Flow and nutrient data collected from FD (upstream) and SWC (downstream) were used to develop a mass balance for a 1.3 km 2nd order reach in the agricultural watershed (Figure 2.2). The reach had three inputs: upstream inflow, multiple 1st order ditches along the reach and groundwater infiltration (both upstream and within the reach). Flow and concentration data measured at the reach outlet (SWC) were used to calculate total mass outputs from the reach. The mass balance consisted of:

$$Q_{SWC}\,C_{SWC} = Q_{up}\,C_{up} + Q_d\,C_d + Q_{GW}\,C_{GW} \qquad (2.1)$$

where Q is discharge ($m^3\ s^{-1}$) and C is concentration (μM); Q_{up} and C_{up} are inputs from the upstream boundary, Q_d and C_d are inputs from the 1st order ditches and Q_{GW} and C_{GW} are inputs from groundwater infiltration.

Due to the similarity in stream characteristics and land use in the agricultural watershed, measured flow and concentration at FD were used to approximate inputs from the additional ditches along the reach. The mass input was adjusted by the proportion of each ditch sub-watershed draining to the reach. First order ditches were ephemeral and only contributed flow and nutrients during storm events.

The only input to the reach during baseflow was groundwater infiltration because no point sources were present in the watershed. Baseflow measured at SWC was used to estimate groundwater contributions since this flowpath was not measured explicitly. Groundwater inputs were separated into two components (upstream and within the reach) by scaling the flow by the proportion of reach length compared to the total flowpath length in the watershed. Upstream concentrations were based on values measured at FD. Since the 1^{st} order ditches were ephemeral, concentrations measured during baseflow at the confluence of FD and the canal reflected upstream inputs only. The concentration of groundwater entering within the reach was assumed to be the same as that measured at SWC during baseflow. Separate reach-scale nutrient uptake experiments (data not shown) were conducted that showed NO_3-N uptake lengths in the canals were >1000 m. This suggests relatively low retention along the reach which supports the use of baseflow concentrations as SWC as the inputs.

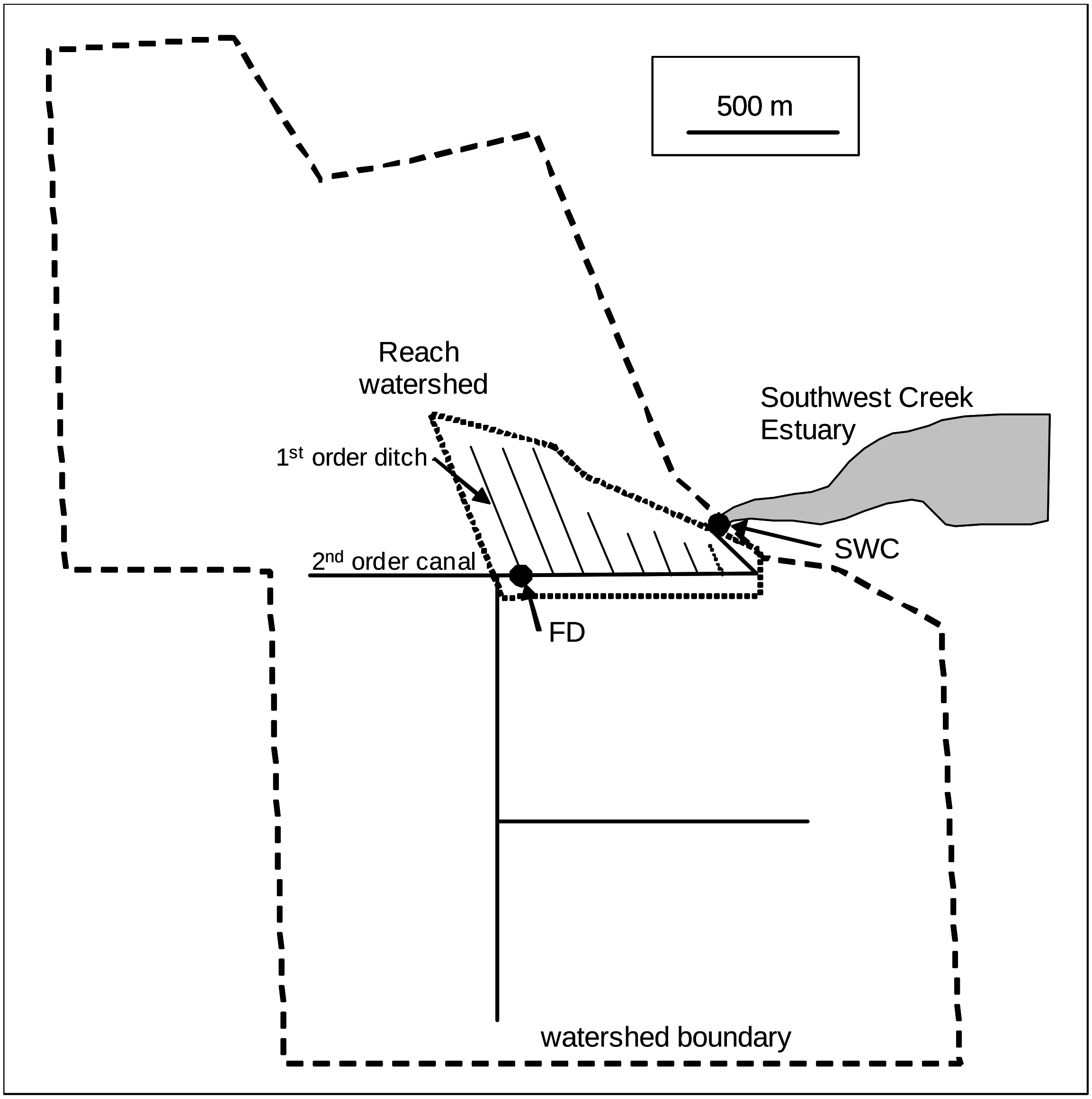

Figure 2.2. Watershed area of the agricultural watershed (SWC), including canals and ditch configuration of the reach for which the mass balance was calculated.

Upstream flow during baseflow was estimated as stated above (the proportion of groundwater infiltration attributed to the upstream reach). During storm events, the upstream flow was determined by the difference in total outputs (measured at reach outlet, SWC) and inputs (ditches and groundwater infiltration). Because of the close proximity of the monitored ditch outlet (FD) and the upstream boundary, concentrations measured at FD were applied to the flow to calculate the mass of nutrients entering the reach during storm flow as well.

These inputs (upstream, ditches and groundwater infiltration) and outputs measured at SWC were used to calculate a reach scale mass balance of NO_3-N, NH_4-N and PO_4-P and estimate the mass of nutrients retained instream.

2.2.5 Reach scale retention

An empirical reach scale uptake model based on mass transfer velocity, V_f, and discharge measured in the 2nd order streams in the agricultural watershed was applied to estimate the percent of the nutrient load retained instream. The model was originally developed during a previous study of this same watershed (Ensign et al. 2006). The lower 1.3 km of the stream network was represented in a spreadsheet model, which predicted exponential decline in nutrient concentration at 10 m intervals. Mass transfer velocities for NH_4-N and PO_4-P were measured using short-term nutrient injection experiments (Webster and Ehrman 1996). Retention metrics based on V_f and discharge were applied to the measured downstream load to estimate the upstream load to the stream network on a daily basis and subsequent instream mass retention. Nutrient uptake was modeled using a modification of Equation 2 (sensu Doyle et al., 2003):

$$C_s = C_{s-1} e^{\frac{-x \times V_f}{d \times u}} \tag{2.2}$$

where C_s is the concentration in a 10 m stream segment, C_{s-1} is the concentration in the adjacent upstream segment, x is the distance between the segments (10 m), d is channel depth and u is stream velocity. For a complete model description and equations, please refer to (Ensign et al. 2006).

The original model was applied to a 5-month period of record from 8/2003 to 12/2003 during which only outputs from the watershed were measured. However, during the 16-month period from 4/2005 to 7/2006, both inputs to the reach and outputs downstream were measured which allowed the model to be used in a forecasting mode. The model was calibrated and robustness was tested by comparing predicted to measured downstream loads.

2.3 Results

2.3.1 Flow and nutrient concentrations

During baseflow conditions, instream nutrient concentrations were greater in the agricultural stream than the silvicultural stream with the exception of NH_4-N, which was similar in both streams (Table 2.1). In the silvicultural stream, median NH_4-N concentrations were lower during storm events, while NO_3-N concentrations were slightly higher which is indicative of a flush of NO_3-N from the terrestrial environmental to the stream. Median NO_3-N and PO_4-P concentrations were significantly greater in the agricultural stream during storm events compared to baseflow, but no significant changes were observed in median concentrations of NH_4-N and DOC.

Table 2.1. Instream nutrient concentrations measured at the watershed outlet in the silvicultural (BA) and agricultural (SWC) watersheds. Median concentrations (25th percentile, 75th percentile) were separated based on baseflow or storm event conditions.

	Silviculture	Agriculture
Baseflow		
NO_3-N (μM)	0.7 (0.4, 1.9)	7.0 (2.8, 27.6)
NH_4-N (μM)	8.6 (3.3, 22.1)	8.5 (4.0, 15.2)
PO_4-P (μM)	0.3 (0.2, 0.5)	1.3 (0.7, 2.9)
DOC (mg/L)	9.6 (7.3, 12.0)	18.7 (15.5, 21.0)
Storm events		
NO_3-N (μM)	1.4 (0.8, 2.4)	54.1 (27.1, 108.6)
NH_4-N (μM)	2.8 (1.8, 4.7)	8.9 (5.9, 15.2)
PO_4-P (μM)	0.3 (0.2, 0.4)	12.9 (4.4, 21.3)
DOC (mg/L)	10.2 (7.9, 14.2)	18.0 (15.7, 23.1)

Hydrographs of representative winter and spring storms are shown in Figures 2.3 and 2.4. Streamflow in these coastal streams increased rapidly during storm events due to low infiltration and few channel obstructions. Both streams exhibited a rapid rise in streamflow in response to increased precipitation suggesting low storage capacity in these coastal catchments.

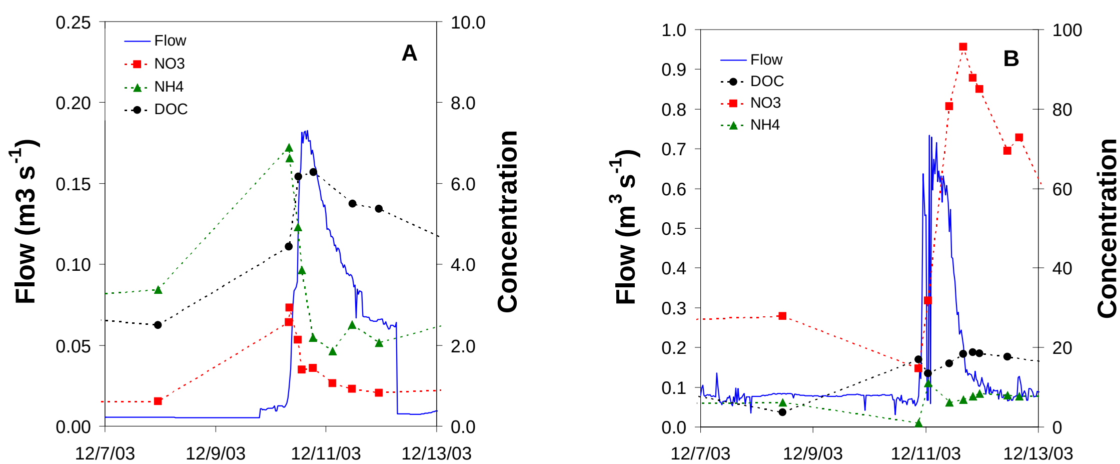

Figure 2.3. Flow and concentration as measured at the outlet of the (A) silvicultural and (B) agricultural watersheds during a winter storm in December 2003. Concentrations of nitrate (NO3) and ammonium (NH4) are in μM; dissolved organic carbon (DOC) concentrations are in mg/L.

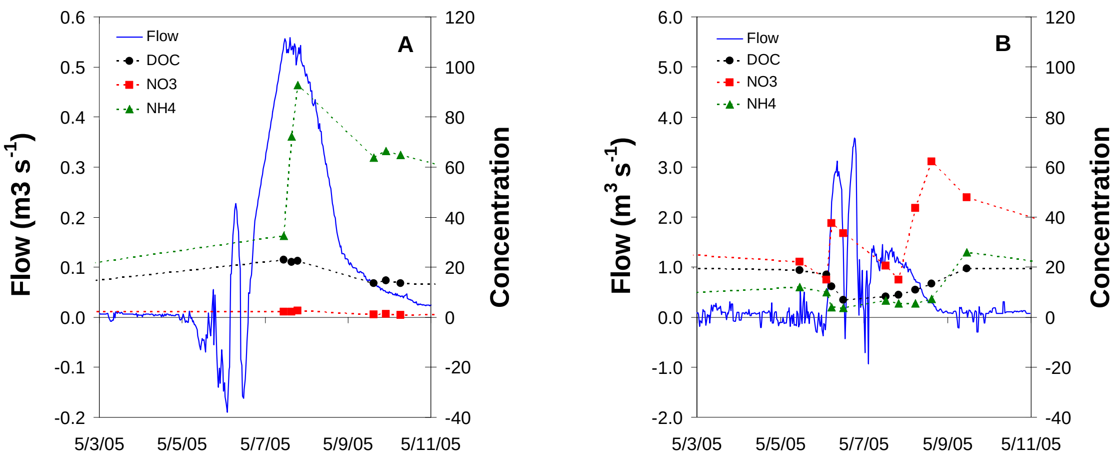

Figure 2.4. Flow and concentration as measured at the outlet of the (A) silvicultural and (B) agricultural watersheds during a spring storm in May 2005. Concentrations of nitrate (NO3) and ammonium (NH4) are in µM; dissolved organic carbon (DOC) concentrations are in mg/L. Increased ammonium concentrations in the silvicultural stream (A) were observed following broadcast fertilization application.

During baseflow, relatively stable concentrations of NH_4-N and DOC were observed. Instream concentrations were diluted by the storm hydrograph as seen as a decrease in concentrations concurrent with increased discharge. In contrast, a concentration pattern in NO_3-N was consistently observed in the agricultural stream during storm events. This concentration pattern was characterized by increased concentrations that essentially mimic the storm hydrograph. Pulses of NO_3-N were observed immediately following peak discharge (Figures 2.3-B and 2.4-B).

Dilution patterns were observed in both DIN species in the silvicultural stream. Since this forested watershed is managed for silviculture, fertilizer application at key times during the tree's life cycle resulted in periodic storm-driven pulses of NH_4-N that were observed during the spring of 2005 (Figure 2.4-A). In contrast to the dilution pattern observed in the agricultural stream, DOC concentrations increased slightly during storm events.

Event-averaged concentrations were compared for rainfall events under dry antecedent conditions (defined as 10 days with no prior rainfall) versus wet antecedent conditions (rainfall <4 days prior). In the agricultural stream, NO_3-N concentrations were significantly greater during storms following dry antecedent conditions compared to wet. Concentrations of NH_4-N, PO_4-P and DOC showed no significant differences (Figure 2.5).

There was a slight effect of antecedent conditions in the silvicultural watershed when event concentrations were averaged over the 3 year study. Concentrations were slightly greater under wet antecedent conditions compared to dry, but differences were not significant (Figure 2.6).

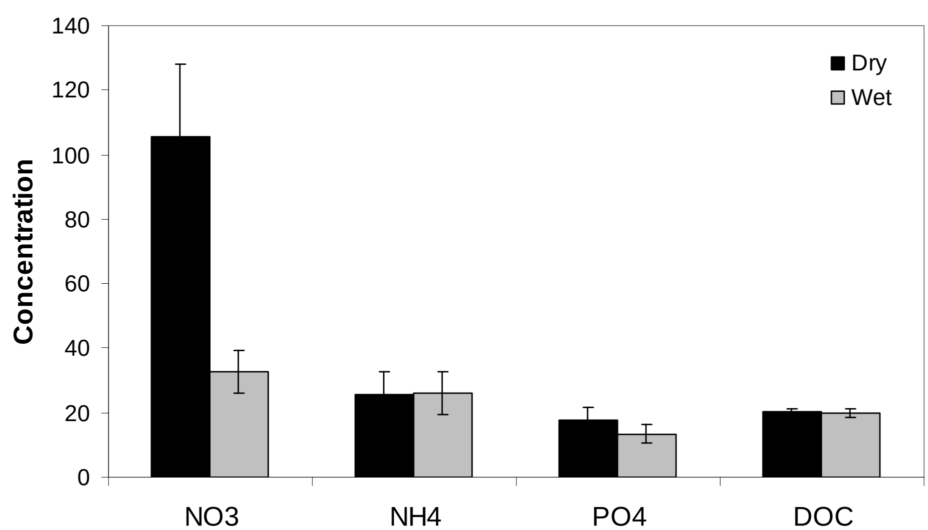

Figure 2.5. Event-averaged concentrations for storms events in the agricultural watershed from August 2003 – July 2006. Concentration units are μM for NO_3-N, NH_4-N, PO_4-P and mg/L for DOC.

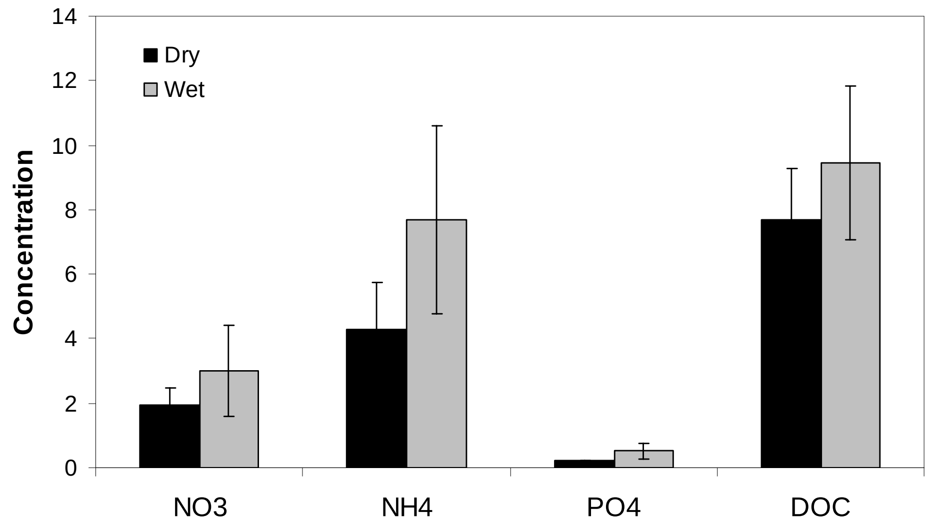

Figure 2.6. Event-averaged concentrations for storms events in the silvicultural watershed from August 2003 – July 2006. Concentration units are µM for NO_3-N, NH_4-N, PO_4-P and mg/L for DOC.

Two periods of low precipitation (9/2004 – 4/2005 and 11/2005 – 7/2006) and one wet period of higher precipitation (5/2005 – 10/2005) were well defined during the study. During the dry periods with low precipitation, antecedent conditions had a significant influence on event-averaged NO_3-N concentrations in the agricultural watershed while no differences were observed in the high precipitation period (Table 2.2). No differences were observed among the other constituents measured in the agricultural stream or the silvicultural stream (data not shown).

Table 2.2. Low and high rainfall periods and the resulting NO_3-N concentrations measured in the agricultural stream. The total number of storms is listed and separated into storms with dry or wet preceding conditions.

	Number of storms (dry/wet)	Average rainfall (cm/month)	Event averaged NO_3-N (µM)	
			Dry	Wet
9/04 – 4/05 (low)	8 (6/2)	6.3	113.1 ± 34.1	34.5 ± 26.0
5/05 – 10/05 (high)	9 (6/3)	21.0	80.0 ± 24.2	57.8 ± 21.7
11/05 – 7/06 (low)	8 (4/4)	5.9	221.8 ± 66.2	58.1 ± 19.1

The event-averaged concentrations were also analyzed for any seasonal or inter-annual effects. In the silvicultural stream, DOC concentrations exhibited a seasonal trend that was also seen in rainfall patterns. Instream DOC concentrations were positively correlated with monthly rainfall ($r^2 = 0.455$, $p < 0.001$). No seasonal trends or correlations were observed in the agricultural stream.

Mass export from both watersheds was measured on a daily basis using flow and concentration data collected at the watershed outlets (Figures 2.7 and 2.8). When loads were normalized to watershed area, export from the agricultural watershed was significantly greater for all solutes measured during the study.

Ammonium was the dominant form of DIN exported from the silvicultural watershed for the entire study period (Figure 2.7). Significant increases in NO_3-N and NH_4-N were observed during FY2. This corresponded to period of elevated rainfall during the spring and summer of 2005 compared to other years and fertilizer application during late summer 2004 and spring 2005. Increased loads of DOC were also observed during FY2 and FY3.

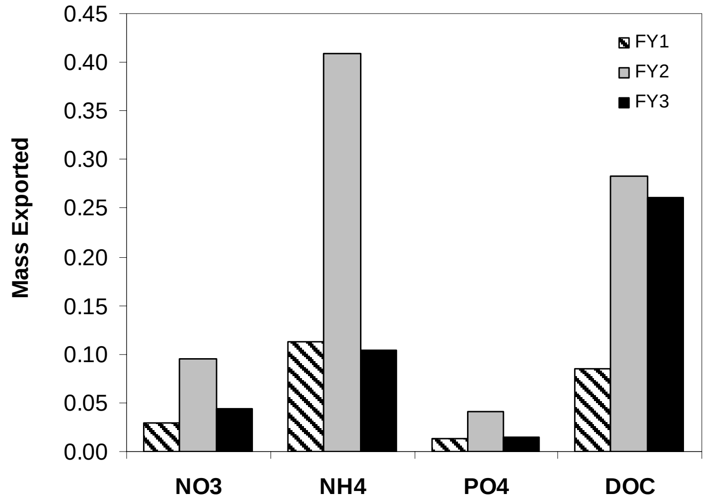

Figure 2.7. Mass of NO_3-N, NH_4-N, PO_4-P (units are kg/ha) and DOC (units are 10^2 kg/ha) exported from the silvicultural watershed from August 2003 – July 2006.

Similar to trends in the concentration data shown in Table 2.1, NO_3-N was the dominant N species exported from the agricultural watershed during the entire study period (Figure 2.8). Export of NO_3-N and DOC were significantly higher during FY2 and FY3 compared to FY1 while NH_4-N was relatively similar throughout the study period. Phosphate export was greatest during FY2, which corresponded to a period of increased spring and summer rainfall.

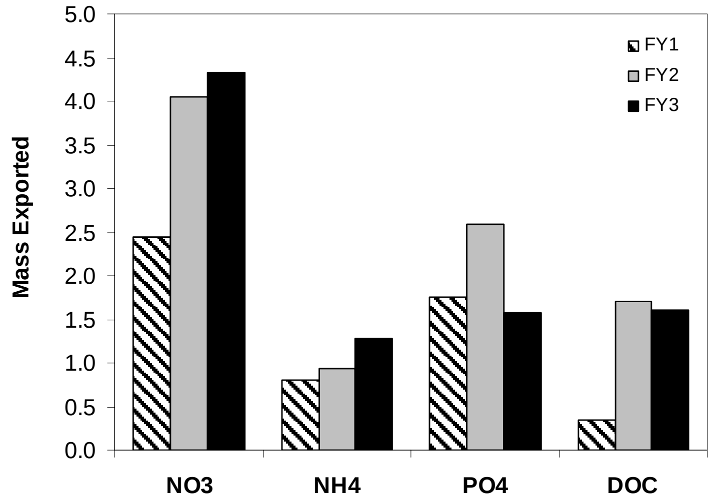

Figure 2.8. Mass per watershed area of NO_3-N, NH_4-N, PO_4-P (units are kg/ha) and DOC (units are 10^2 kg/ha) exported from the agricultural watershed from August 2003 – July 2006.

Cumulative volumetric discharge and inorganic nutrient and dissolved carbon loads were separated into baseflow and stormflow components for the 3 year study period (Figures 2.9 and 2.10). In the silvicultural watershed, 59.4% of the volumetric discharge occurred during storm events (Figure 2.9). Export of DIN and PO_4-P closely followed this pattern (62.8%, 58.1% and 58.4% for NO_3-N, NH_4-N and PO_4-P respectively) while DOC had slightly greater export during storms (71.8%). In contrast, storm events contributed significantly less to the total volumetric discharge (27.3%) in the agricultural watershed (Figure 2.10). Storm-driven export of NH_4-N and DOC closely followed volumetric discharge with 32.5% and 29.8% of NH_4-N and DOC exported respectively. Export of NO_3-N and PO_4-P were more strongly influenced by storm events with 52.0% and 66.7% of the loads exported during storms.

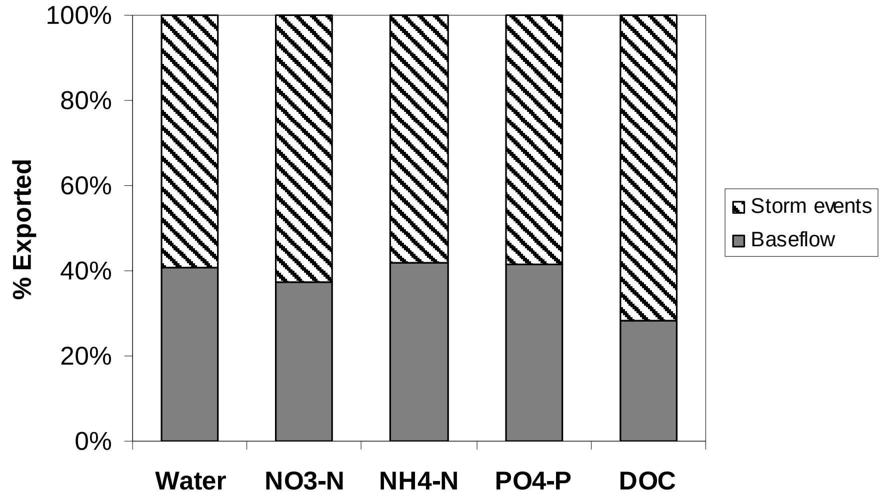

Figure 2.9. Mass (kg) and water volume (m^3) exported from the silvicultural watershed separated into storm and baseflow components; data combined from August 2003 – July 2006.

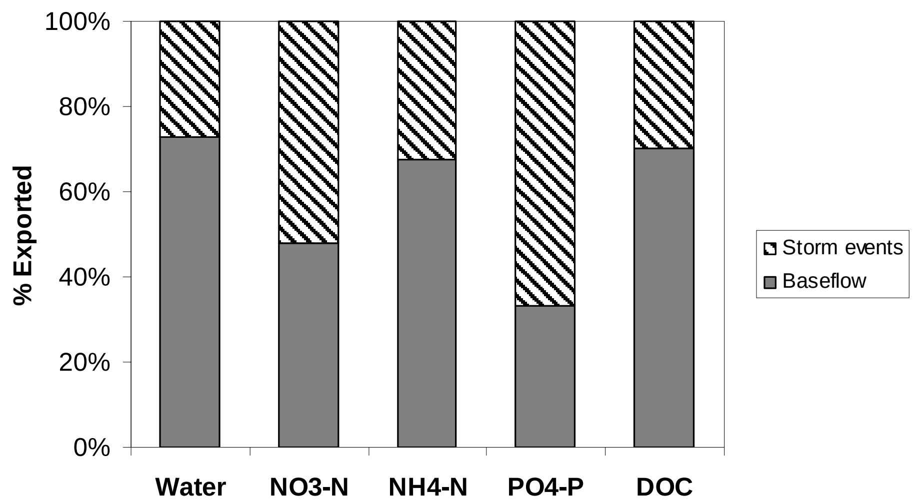

Figure 2.10. Mass (kg) and water volume (m^3) exported from the agricultural watershed separated into storm and baseflow components; data combined from August 2003 – July 2006.

2.3.2 Reach scale mass balance in the agricultural stream

In the agricultural watershed, detailed monitoring data at upstream (FD) and downstream (SWC) monitoring stations along a 2nd order canal was used to calculate total instream retention of NO_3-N, NH_4-N, and PO_4-P over a 16-month period of record (Figure 2.2). Flow and concentration data collected at FD were used to estimate streamflow inputs to the reach and data from SWC were used to estimate outputs.

Total monthly inputs over the study period are shown in Figure 2.11. Peak mass fluxes to the stream were observed during the fall following harvest and during fertilizer application in the spring. As a result of no till farming practices, a majority of the plant biomass remains on the field after harvest for erosion control and nutrient enrichment of the soil. Nutrients are released as this organic material decomposes and are subsequently flushed to receiving waters during storm events. Additionally, fertilizer application (monoammonium phosphate) applied throughout the spring 2006 (February – May) resulted in high fluxes of NH_4-N to the stream. Similar high loads of NH_4-N were not observed in the spring 2005. During 2005, soybeans were the major crop planted and required no N fertilization because symbiotic N fixing bacteria (*Rhizobium sp.*) in root nodules fulfill N requirements of the plant.

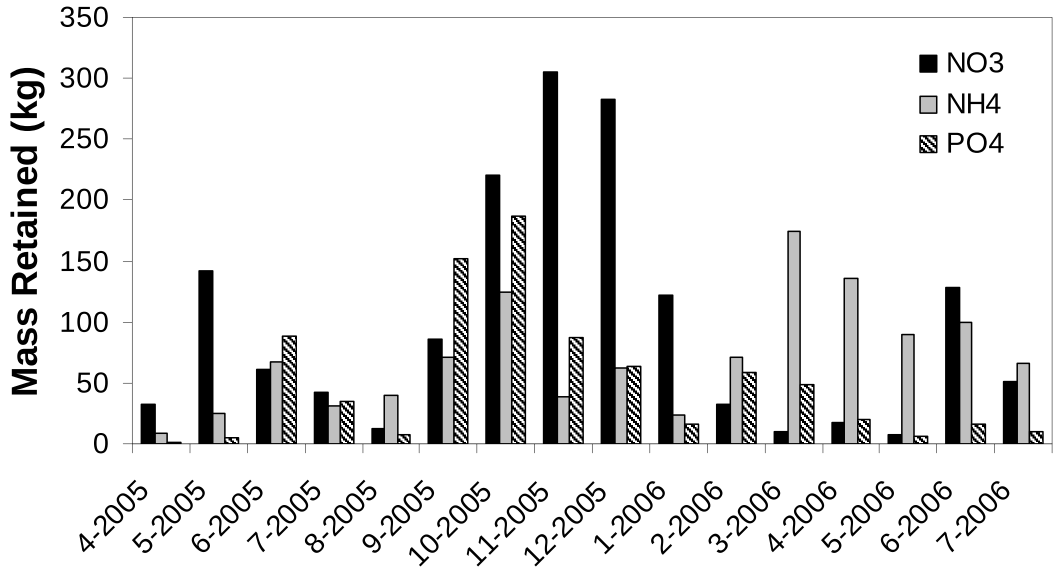

Figure 2.11. Total monthly inputs of nitrate (NO3), ammonium (NH4) and phosphate (PO4). Peak inputs were observed following harvest in the fall and during fertilizer application in the spring.

Results from the mass balance revealed that watershed export of NO_3-N was greater than measured inputs which indicated that the stream appeared to gaining NO_3-N along the reach (Figure 2.12). Separation of these inputs and outputs into baseflow and stormflow components, revealed that the difference between inputs and outputs was greater during baseflow (-49.3% retention) compared to storm events (-33.8%).

In contrast to NO_3-N, instream attenuation of NH_4-N and PO_4-P was significant (46.3% and 13.5% respectively). Separation of total retention into baseflow and stormflow components revealed that instream attenuation was significantly greater during baseflow (50.9% compared to 30.8% retention of NH_4-N during baseflow and storm events respectively; 33.9% compared to -1.7% retention of PO_4-P during baseflow and storm events respectively).

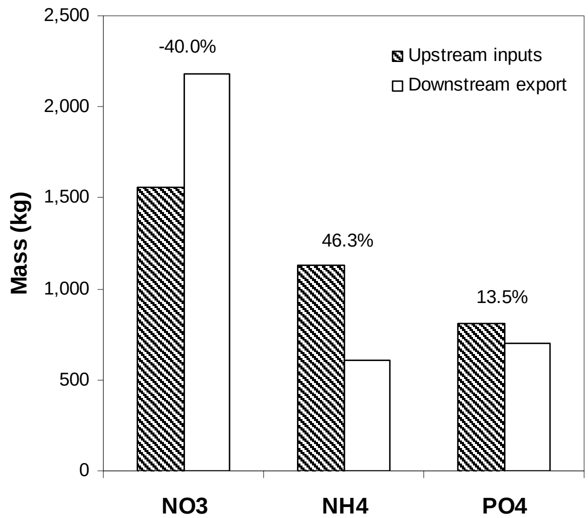

Figure 2.12. Total mass inputs and outputs from the stream reach during the 16-month period of record.

Monthly retention of NH_4-N and NO_3-N during the spring and summer of 2006 is shown in Figure 2.13. High retention of NH_4-N was observed during February – April followed by large losses of NO_3-N from May – July. Fertilizer application occurred from February – May, the same time period with high NH_4-N retention. Large NO_3-N losses in June corresponded to the high precipitation totals during that same period.

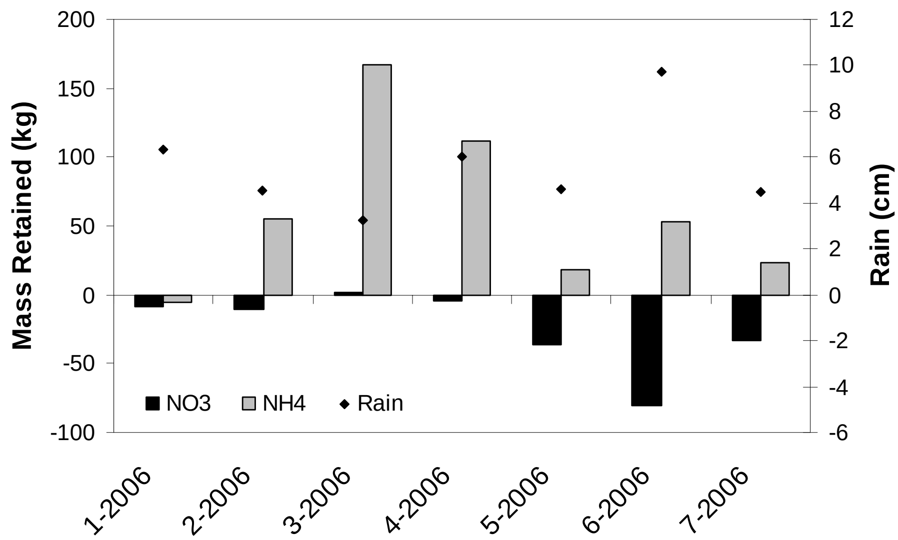

Figure 2.13. Retention of NO_3-N and NH_4-N and monthly precipitation totals.

Instream retention of DIN and PO_4-P for individual storm events was separated by dry and wet antecedent conditions. Retention of NH_4-N and losses of NO_3-N were greater under dry antecedent conditions (Figure 2.14). Comparisons of PO_4-P retention showed no significant differences between dry and wet antecedent conditions.

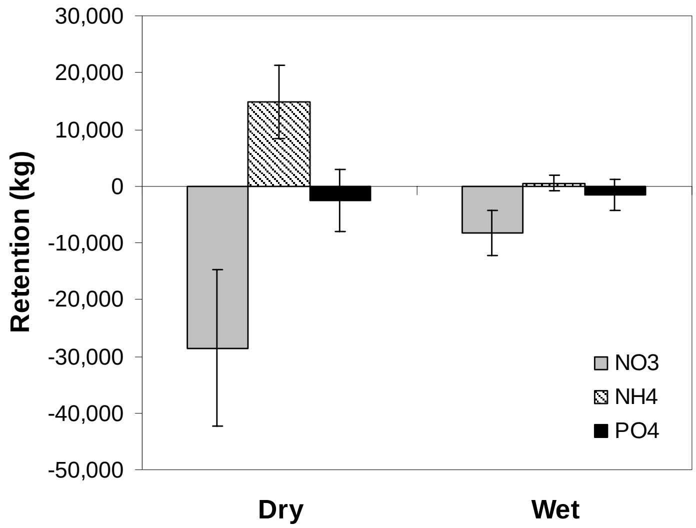

Figure 2.14. Cumulative instream retention of NO_3-N, NH_4-N and PO_4-P during storm events separated by antecedent conditions.

2.3.3 Agricultural stream network model

An empirical model based on V_f and discharge was applied to the agricultural stream network to estimate the percent of the nutrient load retained instream. Retention equations in the model were based on nutrient uptake metrics measured from August – December 2003. Results from the original model application showed that the stream was 100% efficient at retaining nutrients during baseflow (Ensign et al. 2006). Nutrient loads measured at the reach outlet were presumably due to remineralization of organic matter from the streambed. Additional data collected during this study at upstream and downstream stations across multiple seasons allowed for better definition of the effect of remineralization during baseflow on net instream retention. As such, the model was calibrated by reducing remineralization from 100% to 50% for NH_4-N and 65% for PO_4-P.

For the 16-month period that data were collected, the predicted outputs were in general agreement with measured outputs (Figure 2.15). Modeled retention of NH_4-N was 50.9% and the measured retention was 46.3%. Phosphorus retention was over-predicted by the model (31.1%) compared to measured retention (13.5%).

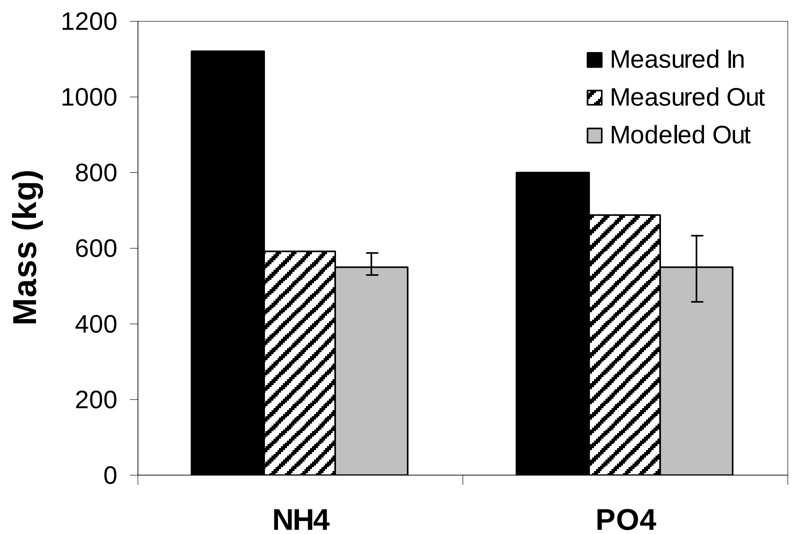

Figure 2.15. Modeled mass export of NH_4-N and PO_4-P compared with measured inputs and outputs from the agricultural watershed.

Analysis of model results on a finer temporal scale (monthly) revealed seasonal patterns in the efficiency of the stream at removing nutrients from the water column (Figures 2.16 and 2.17). High NH_4-N retention was observed during the spring of 2006. This increase was reflected in model results, but the magnitude was under-estimated by nearly 50% during March and 40% during April and over-estimated by 140% during May. Phosphorus retention was lower overall than NH_4-N and more variable on a monthly basis. Similar results for the spring of 2006 were observed with the model under-predicting retention during this time (60%, 50% and 10% during March, April and

May respectively). Net negative retention of both NH_4-N and PO_4-P was observed during the winter months following harvest.

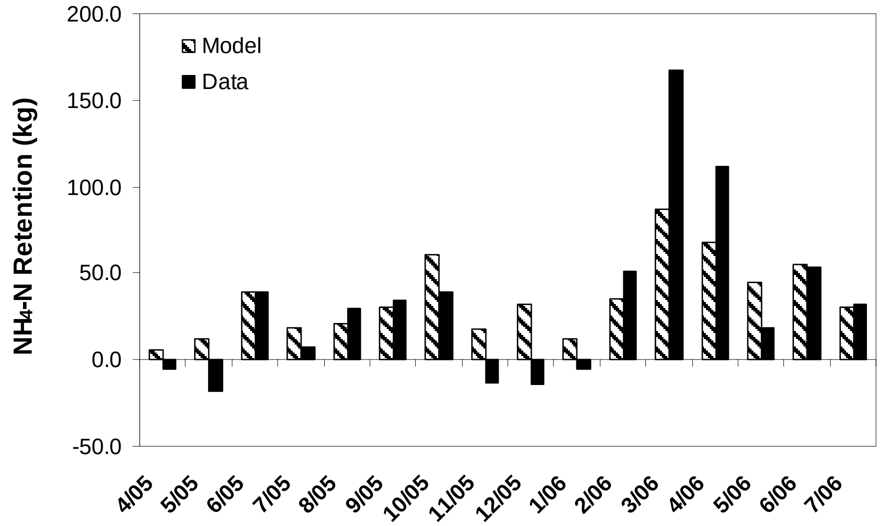

Figure 2.16. Instream retention of NH_4-N based on mass balance analysis (Data) and modeled results (Model).

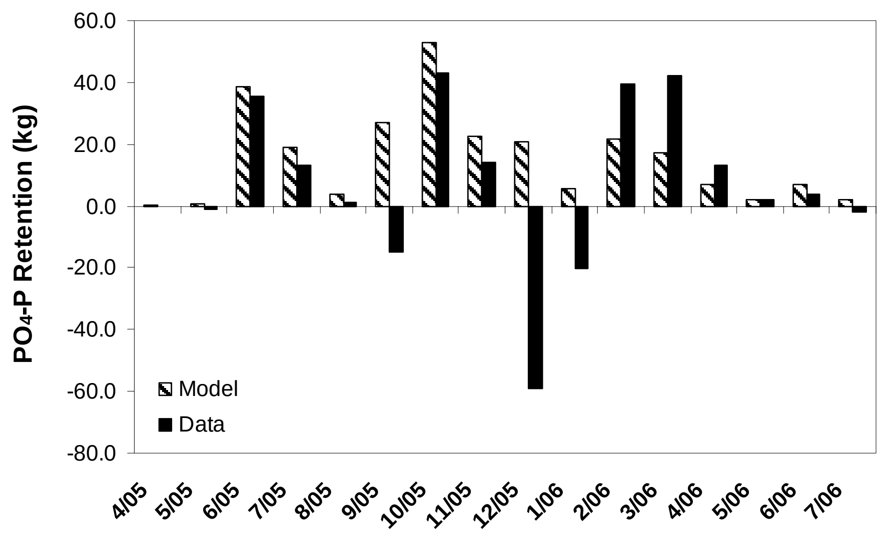

Figure 2.17. Instream retention of PO_4-P based on mass balance analysis (Data) and modeled results (Model).

2.4 Discussion

Natural stream networks in many watersheds in the southeastern coastal plain, including the two that were the focus of this study, have been altered from natural streams into engineered drainage systems. These modifications prevent standing water from accumulating on the surface following storm events and lower the groundwater table to sufficient depths such that plant roots are exposed to aerated soil (Lebo and Herrmann 1998). The flat topography, relatively high water table and close spacing of the drainage ditches allow the drainage system to serve two primary functions: remove water from the land surface quickly during storm events and maintain higher groundwater levels during dry periods by installation of retention structures at watershed outlets.

While functionally important for agricultural and silvicultural productivity, these changes result in a significantly altered hydrology affecting both timing and duration of storm event discharge (Amatya et al. 1996) and nutrient biogeochemistry (Kemp and Dodds 2002a). Rather than slow release of infiltrating precipitation, the hydrograph rises quickly due to reduced infiltration and interception during fallow periods in the agricultural watershed. Peak flows are greater as the same volume of water is released over a much shorter duration. Decreased hydraulic retention time and increased water depth decreases the proportion of the streamwater volume in contact with biologically active streambed sediments, thereby reducing nutrient retention.

2.4.1 Storm flow generation and the flushing response

During periods of minimal rainfall, there was consistently low streamflow in 1st order ditches in both land uses indicating that discharge was largely controlled by storm events. Flat topography of these watersheds allowed formation of stagnant pools in many of these ditches during baseflow periods. Low flows, long retention times, high biomass levels (in the form of plants, algae, leaf litter and detritus) and organic rich sediments resulted in a wetland-like function. In the absence of point sources, the stream itself acts a source of nutrients and carbon via remineralization of organic matter and active recycling among biological communities. Both were intensively recycled resulting in stable instream concentrations.

In the silvicultural watershed, decomposition of pine needles and leaf litter in the stream lead to dissolved humic substances that were relatively recalcitrant compared to algal-derived DOC in the agricultural streams. Algae and submerged macrophytes proliferated in the agricultural streams because of higher light levels due to a limited riparian canopy and elevated nutrient concentrations from fertilizer application. Remineralization of this organic matter resulted in a release of NH_4-N and a more bioavailable form of DOC in the agricultural stream. In both land uses, NH_4-N was the dominant form of DIN during baseflow and DOC concentrations were greater in the agricultural stream.

Streamflow increased rapidly during storm events in these highly modified channels due to decreased infiltration, few channel obstructions and extensive areal coverage of the stream network. The sharp rise in the hydrograph suggests that storm flow is generated by pre-event groundwater that is displaced by rapid movement of precipitation through macropores or discontinuities in the soil matrix.

Prior to rainfall initiation, subsurface flow was predominantly through the lower soil horizons creating oxygenated conditions in the pore spaces of near-surface soils similar to other coastal watersheds (Amatya et al. 1996). Particularly in the agricultural watershed, fertilizer application and organic matter mineralization provided an abundant supply of NH_4-N. The combination of oxygenated terrestrial soils and NH_4-N supply stimulated rates of nitrification allowing NO_3-N to accumulate in near-surface soils. Rising water table intercepts this storage zone of NO_3-N and flushes it to the stream causing concentrations to increase rapidly. This mechanism for the NO_3-N flushing response has been observed in other agricultural and forested catchments following storm events (Creed and Band 1998, Petry et al. 2002, Poor and McDonnell 2007) but has not yet been described in coastal catchments with relatively flat topography.

In the agricultural watershed, peak NO_3-N concentrations occurred after peak discharge. This delay is likely attributed to a combination of precipitation falling directly on the water surface and pre-event groundwater both with low NO_3-N concentrations contributing the first portion of storm flow. Concentrations increased rapidly as NO_3-N was flushed from subsurface soils.

Antecedent precipitation can be a critical controlling factor of N export from terrestrial soils during storm events, particularly in the magnitude of the source of NO_3-N (Biron et al. 1999, Poor and McDonnell 2007). Event averaged nitrate concentrations in the agricultural watershed in this study were significantly greater during storms following prolonged dry periods than during storms following wet periods. Prolonged periods with little rainfall allowed oxygenated conditions to persist in near-surface soils creating ideal conditions for nitrification. During dry periods, greater production of NO_3-N occurred in near-surface soils increasing the terrestrial pool of NO_3-N and storm-driven flushing to the stream. While during wet periods, saturated soils created conditions for rapid stormwater runoff generation which diluted instream concentrations.

In contrast to NO_3-N flushing, a dilution response was observed in NH_4-N concentrations in the agricultural watershed. Instream concentrations were diluted by pre-event groundwater with low NH_4-N concentrations. Antecedent precipitation affected the concentration response during hydrograph recession. During storms following prolonged dry periods, NH_4-N concentrations continued to decrease with hydrograph recession. But in storms with higher antecedent soil moisture, a delayed increase was observed following flow recession. Presumably, the source of NH_4-N from fertilizer application and organic matter mineralization in surface and near-surface soils was unable to be nitrified under saturated conditions and was flushed to the stream via surface overland flow either dissolved or transported attached to eroded soil particles.

The characteristic flushing response was observed in the silvicultural watershed for both NO_3-N and NH_4-N, with peak DIN concentrations preceding peak discharge. This was likely attributed to pre-event groundwater with high DIN concentrations that was quickly displaced by infiltrating precipitation. Instream concentrations peaked prior to peak discharge, but quickly decreased as pre-event water mixed with precipitation with low N concentrations and the DIN source decreased. Except during times of fertilizer application, DIN export in the silvicultural watershed was similar to other forested watersheds showing a rapid concentration decrease. Creed and Band (1998) attributed similar NO_3-N concentration increases to flushing of near-surface soils. Concentration declined as the NO_3-N source decreased and the magnitude of this source was affected by antecedent soil moisture. Other studies (Burns 1998, Iqbal 2002) attributed similar concentration patterns to displacement of high nitrate groundwater by infiltrating precipitation.

While NO_3-N concentration patterns were relatively consistent between the two land uses, DOC dynamics were considerably different. In the silvicultural watershed, DOC concentrations mimicked the hydrograph. Positive correlation between precipitation and concentration revealed that precipitation was a strong predictor of instream concentration. Dissolved organic carbon accumulated in near-surface soils during dry periods before the storm and was flushed to the stream by precipitation infiltration similar to the NO_3-N flushing mechanisms (Boyer et al. 1997, Creed and Band 1998).

In the agricultural watershed, the stream itself functioned as a source of DOC. Dissolved organic carbon accumulated during baseflow in the wetland-like ditches as plant and algal biomass decomposed. Groundwater low in DOC diluted this source of carbon causing concentrations to decrease as discharge increased. Regardless of antecedent soil moisture, DOC concentrations exhibited a delayed increase as the hydrograph was receding. This delay was attributed to sorption of DOC to soil particles, which resulted in a delayed flushing of soil storage zones. Similar delayed flushing of DOC during snowmelt and storm events was observed in other forested catchments (Boyer et al. 1997, Inamdar et al. 2004).

Geomorphic stream features are important regulators of hydraulic residence time while nutrient uptake is primarily dictated by biochemical characteristics. However, these two are intrinsically linked in that retention time indirectly affects biological retention by controlling the amount of water volume exposed to the biologically active sediment communities. Additionally, hydrodynamic transport of streamwater into biological active sediment communities is critical for removal and retention of nutrients.

High NO_3-N concentrations stimulate biological retention, including permanent removal via denitrification, while low retention time increase loads exported. In the agricultural watershed, NO_3-N export was much greater during storm events (greater than 50%) compared to volumetric discharge (approximately 30%), indicating that instream retention was not able to keep up with increased loads. Similar studies have shown that decreased retention despite high rates of denitrification in streambed sediments lead to a large fraction of NO_3-N exported downstream (Bohlke et al. 2004, Royer et al. 2004).

2.4.2 Land use differences

Land use has been well established as a factor affecting instream concentrations and nonpoint source nutrient transport from watersheds (Johnson et al. 1997, Jordan et al. 1997). The percentage of the watershed dedicated to agriculture is especially important in predicting N export (Jordan and Weller 1996, Allan 2004) and instream concentrations (Arheimer and Liden 2000, Kemp and Dodds 2001). Agricultural land use degrades stream integrity by increased nonpoint source nutrient loads, degraded riparian and stream habitat and altered stream hydrology. Landscape metrics, particularly the percentage of agriculture in the watershed and integrity of riparian corridors, explained 65-84% of the variation in yields of N, P and suspended sediment for 78 catchments across the five-state Mid-Atlantic Highlands region (Jones et al. 2001).

Export of DIN and P from the two watersheds in this study supported these conclusions, with the agricultural watershed exporting more than 5 times total DIN (NO_3-N + NH_4-N) compared to the silvicultural watershed. Nutrient export in the agricultural watershed was also shown to be highly dependent on crop management activities, including spring fertilization and fall harvest. Storm events during these key times mobilized this additional nutrient source and resulted in elevated instream concentrations. Biological uptake of NH_4-N and PO_4-P were able to retain a significant portion of this additional nutrient source, while NO_3-N was flushed downstream.

In a review of the influence of anthropogenic sources on watershed N export, Jordan and Weller (1996) found that NO_3-N discharge increased rapidly when watershed outputs exceeded 20 kg N ha^{-1} y^{-1}. Both watersheds were well under this threshold. However, the agricultural watershed averaged approximately 5 kg N ha^{-1} y^{-1} and appeared to have significant influence on instream water quality, especially during storm events. Nitrate export was significantly greater compared to ammonium and phosphate. This was similar to results observed in agricultural catchments in Scotland: NO_3-N export was nearly 20 times greater than NH_4-N (Petry et al. 2002).

In a study of the effects of pine harvest on water quality, Lebo and Herrman (1998) reviewed nutrient exports from other forests and pine plantations. The authors determined that nutrient exports, particularly organic N, were greater for forests in eastern North Carolina that other upland regions. This was attributed to highly organic soils and subsequently high organic N concentrations in streamwater. Total watershed load increased as a result of low infiltration (approximately 1-7% of incoming precipitation). In the silvicultural watershed, nutrient loads closely paralleled volumetric discharge, similar to other studies of modified drainage networks on water quality (Amatya et al. 1998). However, DOC was more strongly influenced by storms, similar to DOC flushing observed in natural forested catchments (Boyer et al. 1997, Inamdar et al. 2004).

Periodic storm events have the potential to influence total solute export regardless of land use (Hinton et al. 1997, McClain et al. 2003, Inamdar et al. 2006). McClain and others (2003) referred to such events as "hot moments", i.e. short periods of time that have a disproportionate influence on catchment hydrologic and solute response. Comparisons of total watershed exports separated into baseflow and storm events revealed that storm flow controlled exports more strongly in the agricultural watershed. Nitrate and phosphate were exported at greater percentages during storms compared to the total volumetric discharge.

2.4.3 Measured instream retention in the agricultural headwater stream

In the agricultural watershed, instream retention of NH_4-N (46.3%) and PO_4-P (13.5%) significantly affected downstream transport, especially during low flow conditions. During dry periods, flat watershed topography and a high water table resulted in a wetland-like environment. Attenuation of NH_4-N and PO_4-P was attributed to high biological activity and increased retention times. A similar mass balance was calculated in an agricultural stream in Sweden (Jansson et al. 1994). Total annual retention was less than 3%, which is significantly lower than total retention in the stream in this study. However, researchers illustrated the importance of retention time on nutrient attenuation, observing that 20-50% of N inputs were retained during low-flow periods during the summer.

In contrast to significant instream NH_4-N retention, net negative retention of NO_3-N indicated that the stream was gaining NO_3-N along the reach. Greater output downstream suggests that there may be missing and/or underestimated sources along the stream reach or within the watershed. The most likely cause is NO_3-N entering the stream via groundwater flowpaths which were not explicitly monitored during this study. Because of little riparian vegetation, high NO_3-N groundwater can flow to the stream unimpeded. This was observed in high sustained peak concentrations of NO_3-N in analyses of storm hydrographs at the watershed outlet.

Separation of the NO_3-N balance into baseflow and storm flow components showed smaller gains along the reach during storm events. While watershed export was greater overall during storms, elevated instream concentrations may have enhanced biological activity, stimulating rates of denitrification and other assimilative retention processes. Reach scale measurement of N attenuating processes in a similar agricultural watershed in the upper Mississippi Basin found denitrification to be important uptake component but that inputs along the stream reach attributed to nitrification and high NO_3-N groundwater resulted in increased NO_3-N concentrations along the stream reach (Bohlke et al. 2004).

Seasonality was observed in the nutrient balance and was attributed to temperature and precipitation variability as well as agricultural management activities, including fertilizer application during the spring, rapid plant growth of the crops during the spring and summer and fall harvest. Elevated instream retention of NH_4-N was observed following fertilizer application during spring 2006. Although this was a period of relatively low cumulative precipitation, the streambed wetted perimeter was elevated due to water management structures at the watershed outlet retaining water in the stream network. During this period, fertilizer application resulted in increased delivery of nutrients to the stream. Increased instream concentrations coupled with warmer temperatures and increased sunlight reaching the water surface contributed to high algal and macrophyte growth in the stream channel, sequestering NH_4-N. This highly bioavailable organic material was quickly decomposed and settled to the sediments resulting in organic-rich, mucky streambed sediments.

During the summer months, water levels decreased exposing these organic rich sediments creating ideal conditions for rapid remineralization and nitrification resulting in a release of NO_3-N from the streambed sediments. This mechanism is supported by high instream NH_4-N retention following fertilizer application (February – April 2006) and subsequent NO_3-N release to the stream during the summer months (May – July 2006). These data suggest the potential for formation of nitrification hot spots in exposed streambed sediments and highlight the need for further measurement of specific biogeochemical processes (mineralization and nitrification).

2.4.4 Modeled nutrient uptake in the drainage network

The nutrient spiralling concept describes the downstream movement of N as it cycles between organic N in biomass and DIN in the water column (Newbold et al. 1981). Numerous reach scale assessments have used this approach to assess instream retention across multiple biomes (Webster et al. 2003, Mulholland et al. 2004). Mass transfer velocity is a nutrient spiralling metric that has been described as a measure of biologic activity independent of hydrologic characteristics and nutrient concentrations (Doyle 2005). As such, it has been used to compare assimilative capacity of streams across ecosystems (Webster et al. 2003).

Instream retention in the agricultural drainage network was modeled by applying the nutrient spiraling framework to data collected at upstream and downstream monitoring stations. During baseflow, the stream was presumed to be at steady state with the net effects of mineralization, assimilation and removal processes reflected in concentration differences between the two sites. A previous application of this model did not account for remineralization during baseflow but rather assumed 100% retention based on results from nutrient injection experiments. Baseflow retention was still modeled as a cumulative process (i.e. individual retentive and removal pathways via assimilation or denitrification were not explicitly defined) but detailed monitoring data across multiple seasons and flow regimes resulted in a more accurate representation of this process. As such, ammonium retention during baseflow was reduced to 50% and phosphate to 65%.

Both the reach mass balance and the watershed model illustrated the importance of instream retention of watershed derived NH_4-N and PO_4-P in the agricultural stream network. Net modeled uptake of NH_4-N and PO_4-P was 47% and 14% respectively compared to measured uptake of 51% and 31% for NH_4-N and PO_4-P respectively. These results agreed well with results from the original model application during the fall of 2003, which concluded that the stream network removed 65% and 37% of the incoming NH_4-N and PO_4-P load respectively (Ensign et al. 2006). Based on these consistent results, the stream network was a significant sink for NH_4-N and PO_4-P leaving this watershed.

Analysis of model results on a finer temporal scale revealed seasonal patterns in the efficiency of the stream in removing nutrients from the water column. The mass balance showed higher retention during spring 2006 compared to model results. Under-prediction of spring retention by the model was likely due to stream uptake metrics utilized in the model were measured during the fall and were applied to the entire year.

Warmer temperatures and higher instream concentrations due to fertilizer application during the spring likely contributed to increased biological activity which was reflected in greater instream retention. Corn was planted during this period with high fertilizer application rates compared to other years during the study. Rapid uptake of this N and P by algae and instream aquatic vegetation created a large pool of biologically availably organic matter for bacterial decomposition. Although V_f is generally considered independent of concentration, chronic changes in nutrient regimes in the water column could lead to changes in the biological community responsible for nutrient uptake.

2.5 Conclusions

This study investigated the relative importance of hydrologic and biogeochemical controls on nutrient retention in engineered drainage systems in two contrasting land uses, agricultural and silvicultural. Detailed monitoring of watershed-derived export of nutrient and carbon loads throughout the study period showed that storm events played a disproportionately large role in delivery of nitrate and phosphate in the agricultural watershed and dissolved organic carbon in the silvicultural watershed.

Although actively managed, the silvicultural watershed displayed nutrient and carbon cycling characteristics similar to those observed in natural forested catchments, particularly in the DOC flushing response. Upon stormflow initiation instream concentrations quickly increased as terrestrial storage of DOC was flushed to the stream. Nitrate export from the agricultural catchment demonstrated a clear dependence on antecedent conditions. Flushing from near-surface soils was an important pathway for NO_3-N delivery to the drainage network in the agricultural watershed. During storms following prolonged periods of low precipitation, both NO_3-N concentrations and storm-driven fluxes were higher due to enhanced nitrification in oxygenated terrestrial soils and greater storage in subsurface zones.

Timing of storm-driven nutrient fluxes was shown to be closely linked to fertilizer application and harvest in the agricultural watershed. Initial sequestration of ammonium from fertilizer application into algal and macrophyte biomass, decomposition and sedimentation to streambed sediments resulted in organic rich sediments in the agricultural stream network. As water levels decreased during summer months, these sediments were exposed creating ideal conditions for remineralization and nitrification, which released nitrate back to the stream. During this transition from spring to summer, the stream was initially retentive of N (as ammonium) and later became a source of nitrate. Further investigation into organic N dynamics and measurement of mineralization and nitrification in these exposed sediments will help determine the link between these two temporally separated processes.

The engineered drainage network in the agricultural catchment is representative of many similar catchments throughout the eastern U.S. coastal plain with high water tables and flat topography. Although these straight channels lacked heterogeneity often deemed critical for nutrient retention (meandering reaches, woody debris, riffle-pool sequences), the reach scale mass balance and application of an empirical stream network model showed significant retention of ammonium and phosphate. Management of these proximate coastal drainage networks to minimize downstream nutrient export is critical in a region where elevated nutrient loads can have a great impact on nutrient dynamics in sensitive estuarine waters. Storm events were identified as critical times for export of both nitrate and phosphate from the agricultural watershed. Incorporating management strategies that maximize retention time (i.e. retention structures at reach outlets) and encourage hydrodynamic transport of nutrient-rich stream water to biologically active sediment communities can further reduce downstream export.

CHAPTER 3:

SPATIAL AND TEMPORAL PATTERNS OF DENITRIFICATION IN COASTAL HEADWATER STREAMS AND THE INFLUENCE OF DENITRIFICATION ON INSTREAM NITRATE REMOVAL

3.1 Introduction

Human activities have dramatically altered the nitrogen (N) cycle by increasing the amount of fixed N via fertilizer manufacturing to levels far exceeding inputs from natural N_2 fixation. This imbalance is reflected in elevated N concentrations in aquatic ecosystems with particularly adverse consequences for sensitive coastal and estuarine waters, including increased rates of primary productivity (eutrophication), reduced biodiversity, habitat degradation and food web alterations (Nixon 1995, Rabalais et al. 2001, Paerl et al. 2002). Extensive ditching and draining of adjacent coastal watersheds has resulted in a stream network consisting of regularly aligned drainage ditches with little geomorphic heterogeneity. The flat topography results in a hydrologically flashy system that experiences rapid peak flows during storm events with very low flow between storms. This has significant implications for nutrient export from these proximate coastal watersheds in that nutrient pulses during storms may quickly enter sensitive estuarine waters at high concentrations.

Low-order streams make up the vast majority of river miles compared to larger rivers and have higher sediment surface to water volume ratios resulting in greater contact of nutrient rich streamwater with biologically active sediments. As such, headwater streams are critical locations for N removal via denitrification. However, anthropogenic modifications of coastal drainage networks (channelization, removal of riparian vegetation, erosion, siltation) decrease streambed heterogeneity and threaten to diminish the retentive capacity of headwater streams. Additionally, flat topography, homogenous streambed sediments and straight channels typical of these coastal drainage networks do little to encourage hydrodynamic transport of high nitrate (NO_3-N) streamwater to sediment denitrification zones.

This study was conducted to assess the potential for denitrification to reduce downstream N transport in agricultural and silvicultural coastal watersheds in eastern North Carolina. My hypothesis was that denitrification rates would be higher in the agricultural streams compared to the silvicultural streams due to elevated nutrient concentrations and that removal via denitrification would significantly affect downstream nitrate export in both watersheds. Denitrification rates were measured on seasonal basis and enrichment experiments were conducted to determine potentially controlling factors. To assess the impact of denitrification on downstream NO_3-N transport, denitrification rates were compared with results of a previously conducted reach-scale mass balance.

3.2 Methods

3.2.1 Site description

The study was conducted from January 2004 through June 2005 in two coastal headwater streams in eastern North Carolina (Figure 3.1). The streams were located in agricultural and silvicultural watersheds, each of which was monitored extensively for water quality, nutrient loadings and volumetric discharge during this same study period.

The streams in this study are low gradient and characterized by flashy hydrology typical of coastal watersheds.

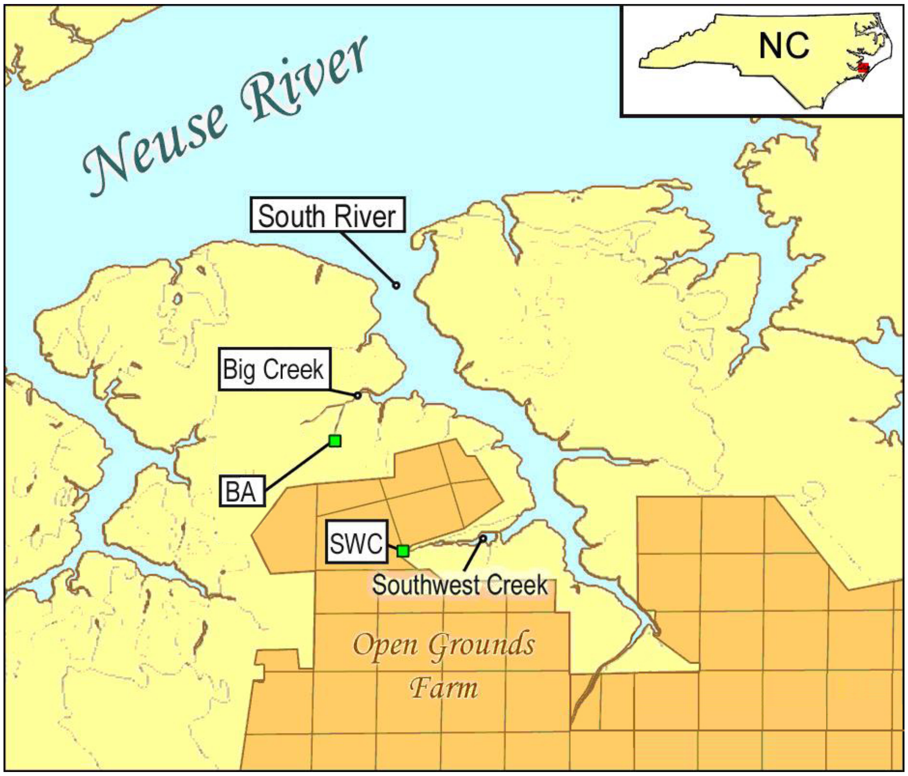

Figure 3.1. Location of the study area in eastern North Carolina, USA.

The agricultural stream is in the Southwest Creek watershed which is located on Open Grounds Farm, a 182 km^2 row crop operation. The watershed is 7.7 km^2 and is planted with corn and soybean crops in an annual rotation. The stream drainage network consists of a regular arrangement of engineered channels which drain to Southwest Creek and subsequently to the South River estuary (Figure 3.2). Water quality was monitored at the watershed outlet (SWC) and at the outlet of a first order ditch (FD). The first order ditches are ephemeral streams with an average width of 0.9 m and depth of 0.2 m during baseflow. During the time of this study the ditch at FD was vegetated primarily with false loosestrife (*Ludwigia* sp.). The surface sediments at FD consisted of organic-rich peat soils while the second order canal (FC) sediment was a coarse to medium grained sand covered by varying accumulations of silt and plant detritus. Canal sediments were regularly reworked during high flow events which covered or scoured submerged vegetation from the stream bed. The canal at FC was approximately 2.6 m wide and 0.2 m deep during baseflow and was heavily vegetated with *Potomogeton pusillus*. The engineered portion of the drainage network is maintained by annual dredging activities that remove woody debris, vegetation and other channel obstructions.

The engineered system of ditches and canals ended at the watershed outlet (SWC). At this site, the creek widen into a natural channel approximately 15 m wide and 1m deep with 5-10 m of riparian vegetation. Stream sediments at SWC were coarse to medium grained sand. The creek site (CS) and immediately adjacent marsh site (CS-M) were located approximately 0.5 km downstream. After initially widening at SWC, the creek narrowed to an incised meandering channel approximately 2 m wide and 1 m deep. Center channel sediments at CS were sandy with accumulation of silt and organic matter along the banks. The marsh site CS-M was a monoculture of black needle rush (*Juncus roemarianus*). During high flow following storm events, streamflow often filled the channel and overflowed into the marsh.

The silvicultural stream is located in a forested watershed managed by Weyerhaeuser Corporation. It is a 2.6 km^2 watershed planted with loblolly pine (*Pinus taeda*) that drains to Big Creek which in turn flows into the South River estuary (Figure 3.2). Flow and water chemistry were monitored at the watershed outlet (BA) throughout the study period. The drainage network is comprised of a series of engineered ditches, similar to the agricultural watershed. However, minimal maintenance activities resulted in ditches with significant accumulation of woody debris, leaf litter and vegetation along the stream banks. The first order ditch (WD) was approximately 1 m wide and 0.1 m deep during baseflow. Channel sediments consisted of a layer of pine needles and leaf litter (typically 5 cm thick) underlain with a light brown clay. The engineered drainage network ended at BA. At this site, channel sediments consisted of a 2-4 cm layer of fine-grained sand with an organic rich peat layer below. At BA, the stream was shaded by a dense riparian canopy that extended 50 m from the stream. The creek (BC) and marsh (BC-M) sites were located approximately 0.5 km downstream from BA. Sediment characteristics and plant community composition at BC and BC-M were similar to the agricultural sites, CS and CS-M.

3.2.2 Denitrification

Sediment samples were collected seasonally for measurement of in situ denitrification on a seasonal basis. Sediment samples were collected for denitrification rate measurements in the agricultural drainage network at 4 stream sites (FD, FC, SWC and CS) and one marsh site (CS-M). In the silvicultural watershed, sediment samples were collected at 3 stream sites (WD, BA and BC) and the marsh site (BC-M) (Figure 3.2).

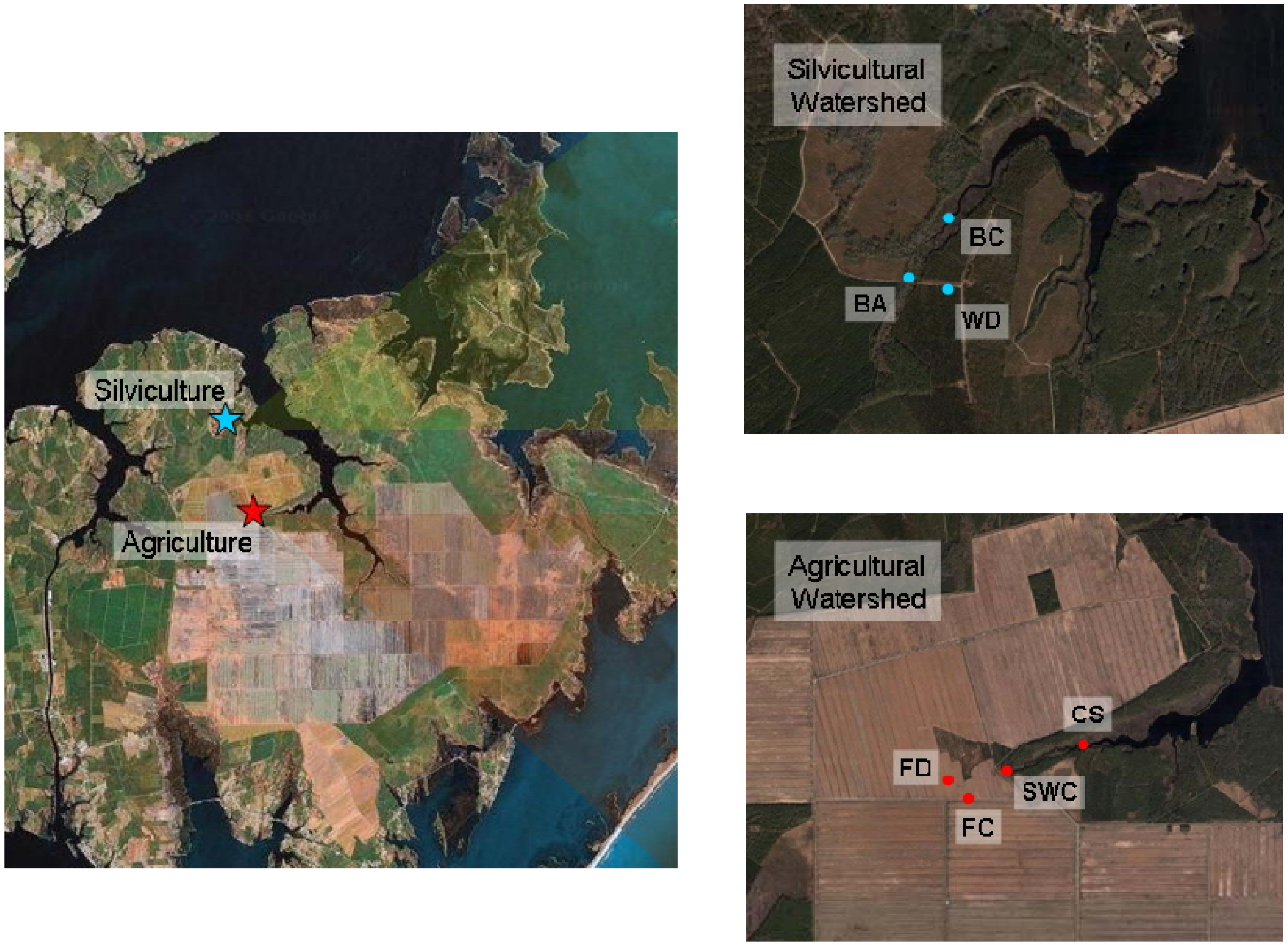

Figure 3.2. Sampling sites for denitrification measurements in the agricultural and silvicultural watersheds.

Rates were measured by removing intact sediment cores (6 cm diameter x 20 cm depth) from the streambed and returning them to the lab for analysis using membrane inlet mass spectrometry (MIMS) (Kana et al. 1994). Cores were collected in triplicate with one water blank per site or treatment. They were kept at in situ temperature while keeping the water column oxygenated for approximately 18 hours prior to measuring rates. At the start of the experiment, cores were capped with gas-tight tops equipped with two sampling ports and a suspended magnetic stirbar to keep the water column well-mixed while not disturbing the sediment surface.

Triplicate water column samples were collected in glass-stoppered test tubes at the initiation of the experiment and every 1-2 hours for a total of 4 time points. During the experiment, total water removed during sampling did not exceed more than 15% of the total water volume in each core. Time series sampling of overlying water was conducted for analysis of dissolved gases (N_2, O_2 and Ar) via MIMS. By using argon as a conservative tracer, small changes in the ratio of N_2:Ar in overlying water can be measured at high precision (0.05%). Denitrification was operationally defined as the net positive flux of N_2 out of the sediment. As such, competing processes such as denitrification and N_2 fixation were not separately quantified if both processes were occurring simultaneously. Separate experiments conducted to measure rates of N_2 fixation in these streams showed negligible rates (data not shown). Sediment rates were corrected for water column processes and other potential methodological errors by subtracting net N_2 fluxes measured in corresponding water blanks. Measurements were made over short time intervals (typically 4-6 hours) to prevent depletion of dissolved oxygen to levels less than 75% of initial concentrations. Oxygen concentrations in the water column were also measured to estimate O_2 flux. Prior to uncapping at the end of the experiment, 50 ml of water was removed for nutrient and DOC analyses.

Experiments were conducted to test the impact of controlling factors on denitrification rates, including changes to water chemistry (elevated NO_3-N and C concentrations) and increased temperature. In experiments with NO_3-N and C additions, cores were incubated in water collected from the site amended with $NaNO_3$ and mannitol respectively and held overnight at in situ temperature for approximately 18 hours. In experiments with temperature variations, rates were measured at *in situ* temperature, allowed to re-equilibrate with site water overnight, and then re-run at the elevated temperature.

An experiment was conducted in the agricultural stream to investigate the potential for NO_3-N saturation. Duplicate sediment cores were collected and amended with progressively increasing nitrate (as $NaNO_3$) ranging from 50 uM to 1000 uM. Similar to other experiments, the sediment cores were held overnight at *in situ* temperature in oxygenated water. Nitrate was added immediately prior to capping the core tubes.

3.2.3 Water chemistry

Water samples were collected at the beginning and end of each denitrification experiment to measure nutrient and dissolved organic carbon (DOC) fluxes. Samples were filtered through Whatman GF/F glass fiber filters (25mm diameter, 0.7 μm nominal pore size) and the filtrate was analyzed with a Lachat Quick-Chem 8000 automated ion analyzer for NO_3-N, NH_4-N, PO_4-P and total nitrogen (TN) concentrations using standard protocols (Lachat Instruments, Milwaukee, WI, USA: NO_2/NO_3 Method 31-107-04-1-A,

NH_4 Method 31-107-06-1-A, PO_4-P Method 31-115-01-3-G, and TN Method 31-107-04-3-B). Dissolved organic nitrogen (DON) concentrations were calculated as the difference between TN and dissolved inorganic nitrogen (NO_3-N + NH_4-N) concentrations. Dissolved organic carbon concentrations were made using a high temperature combustion technique on a Shimadzu model TOC-500, equipped with an ASI-5000A autosampler.

3.2.4 Sediment characteristics

Sediment samples were collected immediately adjacent to each core collected for denitrification measurements with 1 cm diameter syringe corers. Sediment cores were dried for 24 h at 60°C, ground with mortar and pestle, fumed for 8 h with 12N HCl to remove inorganic C, and re-dried. Fumed sediment samples were analyzed for organic C and N content with a Perkin Elmer CHN analyzer (Model 2400 Series II) standardized with acetanilide.

3.2.5 Denitrification rate as a stream reach uptake component

Using nutrient spiraling metrics, stream reach uptake rate (U) is defined as the area specific flux of nutrient uptake characteristic of the stream under unenriched conditions (Stream Solute Workshop 1990, Doyle et al. 2003). Reach scale uptake integrates the effects of advective transport, uptake (both biotic and abiotic) and remineralization. Of these, denitrification is one component that permanently removes NO_3-N from the stream network. To estimate the importance of instream denitrification as a mechanism for NO_3-N retention, rates were expressed in units of flux (mass NO_3-N length^{-2} time^{-1}) and substituted for the uptake rate (U_{den}). Mass transfer velocity ($V_{f,den}$) was then calculated from U_{den} using the following equation:

$$V_{f,den} = \frac{U_{den}}{C}$$

where $V_{f,den}$ is the removal attributed to denitrification, U_{den} is the denitrification rate measured in sediment cores and C is the instream concentration.

To compare the influence of denitrification on instream retention and removal, a loss rate, $-k$, was calculated as:

$$-k = \frac{V_{f,den}}{d}$$

where d is stream depth and $-k$ is expressed as proportion removed per day.

3.2.6 Calculations and statistics

Linear regressions were performed to relate denitrification rates to chemical characteristics of the water column (dissolved nutrient and carbon concentrations) and fluxes of relevant constituents (including nutrients, carbon and oxygen). Significant relationships were determined using a one-way ANOVA with post-hoc comparison of means using Tukey's Honest Significant Difference. All statistical analyses were conducted using R statistical computing software (R Development Core Team 2007).

3.3 Results

3.3.1 Water quality

Instream water quality was strongly influenced by storm events in the agricultural stream. Nitrate concentrations increased quickly in response to increased streamflow. Median baseflow concentrations were 7.0 μM compared to 54.1 μM during storm events, while other forms of N had lower concentrations and were not as affected by storm dynamics (see Table 2.1). Peak concentrations during storm events were considerably lower than other agriculturally influenced streams (Royer et al. 2004, Bernot et al. 2006). Median DOC concentrations were approximately 18.0 mg/L during both storm events and baseflow. These values were within the typical range of other coastal streams (Koetsier III et al. 1997) and generally higher than upland agriculturally influenced streams (Royer and David 2005).

Water chemistry in the silvicultural stream was dissimilar to other natural forests in that NH_4-N dominated the DIN pool (Tank et al. 2000, Hamilton et al. 2001, Dodds 2002). Median concentrations of DOC (9.6 mg/L during baseflow and 10.2 during storm events) were lower than the agriculturally influenced stream. Dissolved organic carbon concentrations were higher than upland forested catchments (Sobczak and Findlay 2002, Strauss and Lamberti 2002) but within the range of other coastal streams (Koetsier III et al. 1997).

3.3.2 Sediment characteristics

Seasonal measurements of organic matter in streambed sediments are shown in Figure 3.3. In the first order stream (FD) in the agricultural watershed, low values were observed in the winter and spring due to ditch maintenance activities which included dredging and clearing of vegetation. Bed scouring during high streamflow events resulted in lower organic matter in the second order stream (FC) compared to FD with the exception of summer 2004, during which high C content in the sediment was likely due to increased production of benthic microalgae. In the forested watershed, there was a consistent pattern of lower organic matter downstream (BA) compared to upstream (WD) due to scouring of sandy streambed sediments at the downstream site.

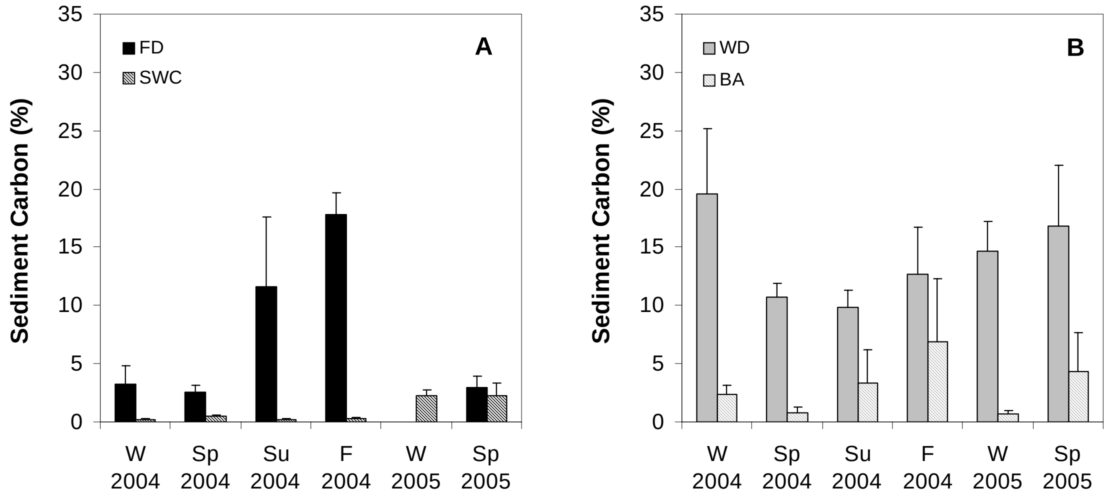

Figure 3.3. Sediment carbon (%) measured in first and second order stream sediments in the agricultural (FD and FC) and forested (WD and BA) watersheds.

3.3.3 Impact of land use on denitrification

Denitrification rates were measured on a seasonal basis from January 2004 through April 2005 (Figure 3.4). Rates were highest in the spring compared to other seasons and generally higher in the 1st order ditches (FD and WD) compared to the 3rd order sites (BA and SWC).

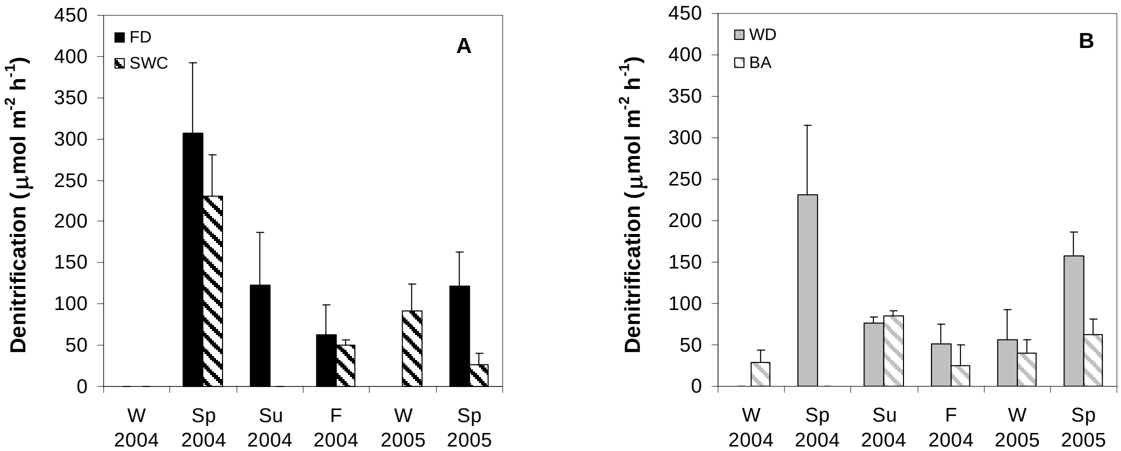

Figure 3.4. Seasonal denitrification rates (μmol m^{-2} h^{-1}) measured in first and second order stream sediments in the agricultural (FD and FC) and silvicultural (WD and BA) watersheds.

Denitrification rates were averaged by site to quantify differences as a function of stream order (Figure 3.5). Denitrification was generally higher in the agricultural drainage network compared to the silvicultural network. Among the agricultural sites, significant differences were observed between CS-M (M) and FC (2^{nd} order stream) and CS-M and SWC (3^{rd} order stream) ($p < 0.05$). The silvicultural stream sites exhibited less variability with the only significant difference between WD (1^{st} order stream) and BA (3^{rd} order stream) ($p < 0.05$). Although not statistically significant, denitrification rates measured at the 1^{st} order sites were similar to those measured at the marsh sites.

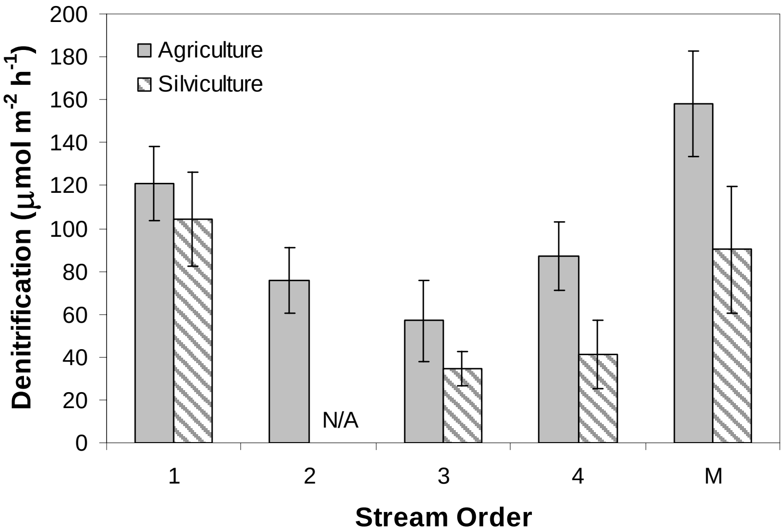

Figure 3.5. Denitrification rates (μmol m^{-2} h^{-1}) as a function of stream order (1-4) and the marsh site (M) adjacent to the 4^{th} order stream. Data were averaged from all seasonal sampling events with error bars indicating one SE. See text for significant differences.

Data were averaged from all sites sampled throughout the study period to test the effect of land use on in situ denitrification (Figure 3.6). Denitrification rates measured in the agricultural stream network (99.3 ± 9.0 μmol m^{-2} h^{-1}, range of 0 – 443 μmol m^{-2} h^{-1}) were significantly higher than the silvicultural network (72.6 ± 10.3 μmol m^{-2} h^{-1}, range of 0 – 362 μmol m^{-2} h^{-1}) ($p < 0.05$).

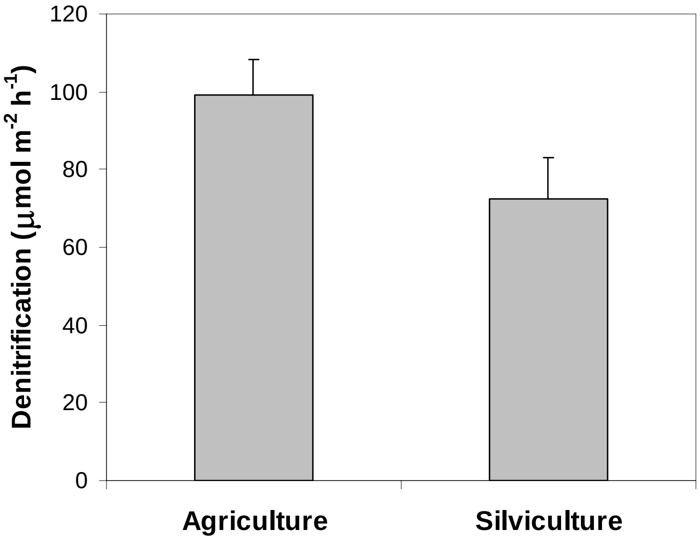

Figure 3.6. Denitrification rates (μmol m^{-2} h^{-1}) were significantly different in the agricultural streams compared the silvicultural streams ($p < 0.05$). Data were averaged from all sites and all seasonal sampling events with error bars indicating one SE.

3.3.4 Environmental controls on denitrification

During each seasonal sampling event, several biochemical measurements were made in addition to denitrification rates, including nutrient (NO_3-N, NH_4-N, PO_4-P and TN) and DOC concentrations and fluxes, oxygen flux and sediment characteristics. Linear regressions were performed between denitrification and each of these parameters to elucidate potential controlling factors. Of these, only three were determined to be statistically significant: sediment % C ($p < 0.01$), sediment O_2 flux ($p < 0.001$), and water blank O_2 flux ($p < 0.05$).

Because of high environmental variability in seasonal denitrification rate measurements, enrichment experiments were conducted to better define and characterize controlling factors on denitrification rates. The effects of temperature and NO_3-N were investigated in 1st order ditches (FD and WD) in both land uses in April 2004 (Figure 3.7). Nitrate was added to each core to simulate concentrations that would be observed during a typical storm event (100 μM in the agricultural stream and 30 μM in the silvicultural stream). Temperatures were selected to represent spring (16 ºC) and summer (25 ºC) conditions. Nitrate stimulated rates above unamended controls with the effect more pronounced when combined with increased temperatures. Significant differences were observed when both NO_3-N concentrations and temperatures were increased ($p < 0.05$). A similar experiment in the silvicultural stream showed no significant stimulatory effect of increased NO_3-N concentrations or elevated temperatures.

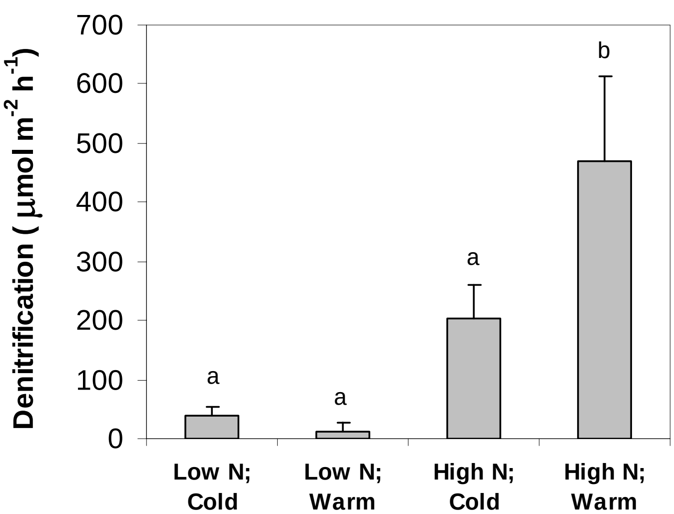

Figure 3.7. Denitrification rates (μmol m^{-2} h^{-1}) after manipulation of temperature and NO_3-N in agricultural sediments. Letter designations show significant differences among treatments ($p < 0.05$). Error bars indicate one SE.

The cumulative effect of organic C and NO_3-N on denitrification rates was investigated in 1st order ditches (FD and WD) in both land uses in September 2004. Initial NO_3-N and DOC concentrations are in Table 3.1.

Table 3.1. Initial concentrations of NO_3-N and DOC in controls and amended sediment cores taken from the agricultural stream (FD). Nitrate was added as $NaNO_3$ and DOC as mannitol.

Treatment	NO_3-N (μM)	DOC (mg/L)
Control	2.82	23.96
C	1.61	81.97
N	97.86	27.21
CN	96.43	76.34

In sediment cores taken from the agricultural stream, NO_3-N increased denitrification relative to unamended controls and DOC treatments in cores (Figure 3.8). Rates were significantly higher in N (337 ± 1.0 μmol m^{-2} h^{-1}) compared to Control (64.8 ± 36.7 μmol m^{-2} h^{-1}) and C (12.3 ± 7.2 μmol m^{-2} h^{-1}) ($p < 0.01$). Denitrification was slightly lower when NO_3-N and DOC were added together although not statistically significant. Concurrent measurement of NO_3-N fluxes showed flux of NO_3-N in excess of denitrification requirements in N-amended cores. Under ambient conditions, NO_3-N fluxes were negative indicating that denitrification may have been supported by nitrification. Oxygen flux measurements were highly variable and showed no statistical differences among treatments (data not shown). A similar experiment at WD in the silvicultural stream showed no significant influences of DOC or NO_3-N.

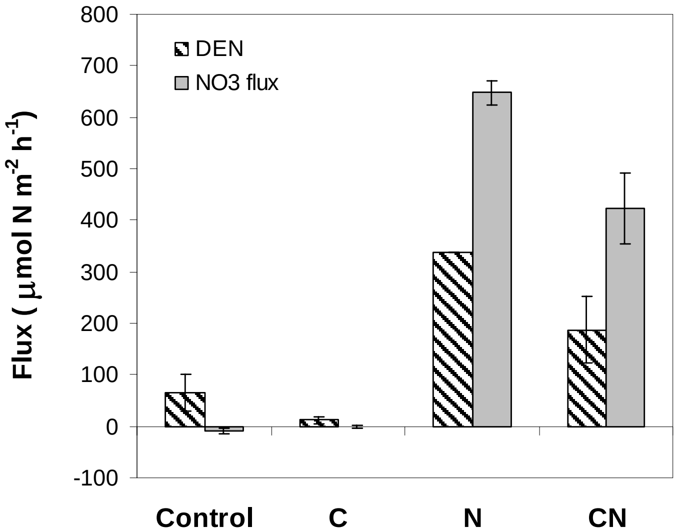

Figure 3.8. Denitrification rates (DEN) and NO_3-N fluxes (NO3 flux) in sediment cores from the agricultural stream (FD) amended with DOC and NO_3-N. Error bars indicate one SE.

Since NO_3-N had a consistent stimulatory effect on denitrification rates at the agricultural sites, the potential for NO_3-N saturation of denitrification was investigated. Cores were amended with increasing concentrations of NO_3-N and exhibited a linear relationship with denitrification rates ($R^2 = 0.997$, $p < 0.001$) (Figure 3.9).

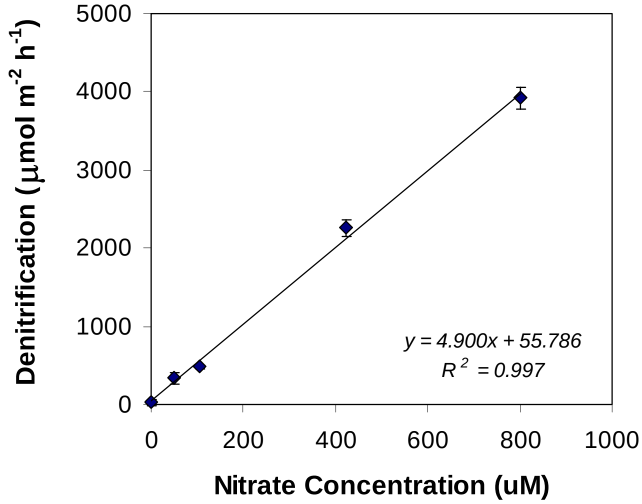

Figure 3.9. Denitrification rates as a function of nitrate concentration in agricultural sediments at FD. Error bars indicate one SE.

3.3.5 Nutrient spiraling metrics

Average seasonal denitrification rates measured in the agricultural stream were converted to nutrient spiraling metrics to assess the potential for denitrification to remove instream NO_3-N (Table 3.2). Denitrification rates were expressed as a positive flux of NO_3-N and converted to $V_{f,den}$ to determine potential for NO_3-N removal relative to instream concentrations. Values of $V_{f,den}$ were highest in the 1st order stream (2.21 mm min^{-1}) but differences were not significant because of high variability among sites.

Nitrate loss rate (-*k*) represents the potential mass of NO_3-N lost to denitrification per day. Values of -*k* were considerably higher than those published for other headwater streams with most sites having >100% loss per day (Alexander et al. 2000, Royer et al. 2004). There were no significant differences among sites or seasons (data not shown).

Table 3.2. Denitrification rates, NO_3-N concentrations, mass transfer velocities ($V_{f,den}$) and loss rates ($-k$) in the agricultural stream. Rates were measured under baseflow conditions during seasonal sampling events. Range of all values is ± one SE.

Site	Denitrification (μmol m^{-2} h^{-1})	NO_3-N (μM)	$V_{f,den}$ (mm min^{-1})	$-k$ (% d^{-1})
FD	120.9 ± 17.2	8.4 ± 2.0	2.21 ± 0.55	>100%
FC	75.9 ± 15.3	9.4 ± 2.6	1.72 ± 0.93	>100%
SWC	56.9 ± 18.8	17.2 ± 4.1	0.39 ± 0.17	57 ± 24%
SC-8C	87.3 ± 16.1	19.3 ± 5.8	1.35 ± 0.67	>100%

3.4 Discussion

Denitrification rates have been shown to vary considerably among aquatic ecosystems (Seitzinger 1988). Denitrification is controlled by complex interactions among physical, chemical and biological factors and is therefore susceptible to changes in environmental conditions due to differences in land use and climate. Primary regulatory factors include the availability of NO_3-N, C, O_2 penetration into the sediments, temperature and soil properties. Understanding these controlling factors is challenging because they vary significantly in both time and space.

Multi-site comparisons of denitrification rates are hindered by differences in methodology. Many laboratory scale studies have been conducted using the acetylene inhibition technique which has the advantage of being inexpensive and relatively simple to use compared to other methods, but can significantly underestimate rates in some situations (Seitzinger et al. 1993). In environments where nitrification contributes a significant supply of NO_3-N for denitrification, rates are under-estimated because acetylene effectively blocks nitrification as well. However, in streams with high NO_3-N concentrations (generally >10 μM) this method was shown to be acceptable for estimating in situ denitrification (Rudolph et al. 1991). Recent studies using the acetylene inhibition technique have added chloramphenicol to inhibit new synthesis of denitrifying enzymes thereby more accurately representing in situ rates (Schaller et al. 2004, Inwood et al. 2005). Bernot and other (2003) measured denitrification in estuarine sediments and obtained comparable results using this technique and MIMS. More recently, MIMS has been used because of its relatively benign impact on sediment biogeochemistry since rates can be measured in whole cores without addition of inhibitors or isotopically-labeled NO_3-N (Cook et al. 2004, Smith et al. 2006).

Denitrification rates measured in this study were within the range measured in other studies of denitrification in stream sediments (Table 3.3). Nitrate concentrations were considerably lower in the agricultural drainage network in this study than other agricultural streams (Christensen et al. 1990, Jansson et al. 1994, Garcia-Ruiz et al. 1998a). Peak instream NO_3-N concentrations during storm events (approximately 200 μM) were 2-3 orders of magnitude lower than peak concentrations in other agriculturally influenced streams. This was also reflected in denitrification rates that were on the lower end of the range of values reported in the literature. Rates in other studies were generally < 400 μmol m^{-2} h^{-1} with considerably higher rates corresponding to peak NO_3-N concentrations (Table 3.3). Nitrate concentrations and denitrification rates measured in the silvicultural stream were similar to natural forested streams (Cooper and Cooke 1984, Seitzinger 1994). Forested streams generally exhibited low rates of denitrification which were also correlated with NO_3-N concentrations (Seitzinger 1994, Mulholland et al. 2004).

Table 3.3. Comparisons of denitrification rates measured in stream and river sediments.

Location	Watershed land use	Denitrification ($\mu mol\ m^{-2}\ h^{-1}$)	NO_3-N (μM)	Method[1,2,3,4]	Reference
Laboratory-scale measurements					
Gelbaek, Denmark	Agriculture	100 – 1400	285 – 930	AIT (cores)	Christensen et al. 1990
Bremer River, Australia	Mixed			MIMS	Cook et al. 2004
	Summer	162	38-99		
	Winter	153	136-154		
Headwater stream, New Zealand	Forest	14.3 – 364	3.3 – 36.6	AIT (slurry)	Cooper and Cooke 1984
	Pasture	364 – 2120	30.1 – 145		
River Wiske, UK	Agriculture	99 – 2586	164 – 2285	AIT (cores)	Garcia-Ruiz et al. 1998a
Yorkshire Ouse, UK	Mixed			AIT (cores)	Garcia-Ruiz et al. 1998b
	upstream	117 – 369	136 – 350		
	midstream	275 – 303	250 – 257		
	downstream	166 – 575	207 – 250		
Duffin Ck, Ontario, Canada	Forest and Agriculture	10 – 125	5	NO_3-N flux (cores)	Hill 1979
Sycamore Ck, AZ	Desert shrub	3-13	0.03	AIT (cores)	Holmes et al. 1996
Kalamazoo River Watershed, MI	Agriculture	223.0	314.3	AIT + C (slurry)	Inwood et al. 2005
	Forest	43.0	14.3		
	Suburban	69.0	28.6		
River Raan, Sweden	Agriculture	14 – 3286	214 – 285	AIT (slurry)	Jansson et al. 1994
Kings Ck, KS	Prairie	0.3	0.4	AIT (slurry, biomass weighted)	Kemp and Dodds 2002a
	Agriculture	1.2	36.4		

Location	Watershed land use	Denitrification ($\mu mol\ m^{-2}\ h^{-1}$)	NO_3-N (μM)	Method[1,2,3,4]	Reference
Shane Ck, KS	Prairie Agriculture	0.4 1.2	1.9 55.3	AIT (slurry, biomass weighted)	Kemp and Dodds 2002a
Jutland, Denmark	Agriculture	450	330	AIT (cores) & microsensor profiles	Nielsen et al. 1990
Swale-Ouse river system, UK	Mixed upstream downstream	 0 – 20 100 – 880	 0 – 10 10 – 60	AIT (cores)	Pattinson et al. 1998
East-central, IL	Agriculture	<7 – 1070	42 – 1130	AIT + C (slurry)	Royer et al. 2004
Big Ditch, IL	Agriculture	0 – 1130	2.1 – 1264	AIT + C (slurry)	Schaller et al. 2004
Potomac River, MA	Mixed	210 – 232	> 14	N2 flux (cores)	Seitzinger 1988
Skit Brook, NJ	Forest	< 20	1	N2 flux (cores)	Seitzinger 1994
Hammonton Ck, NJ	Agriculture	250 – 450	130	N2 flux (cores)	Seitzinger 1994
Sugar Ck, IN Iroquois River, IN	Agriculture	0 – 4400 0-1300	20-1000	MIMS & IRMS	Smith et al. 2006
Culvert Ck, NC	Agriculture potential est. *in situ*	 42 - 210 <1 – 25	 20 – 300 20 – 300	AIT (slurry)	Thompson et al. 2000
Southwest Ck, NC	Agriculture	0 – 443	0 – 51	MIMS	This study
Big Creek, NC	Silviculture	0 – 362	0 – 3	MIMS	This study
Field-scale measurements					
Sugar Ck, IN	Agriculture	120	71	Reach-scale $^{15}NO_3^-$ tracer	Bohlke et al. 2004
Iroquois River and Millstone River, NJ	Mixed (urban and agriculture)	1900 – 15810	> 300	Whole stream MIMS	Laursen and Seitzinger 2002
South Platte River, CO	Mixed	7900	436	Whole stream MIMS	McCutchan et al. 2003

Location	Watershed land use	Denitrification (μmol m^{-2} h^{-1})	NO_3-N (μM)	Method[1,2,3,4]	Reference
Walker Branch, TN	Forest	12	1.6	Reach-scale $^{15}NO_3^-$ tracer	Mulholland et al. 2004
South Platte River, CO	Mixed	6280	286	Mass balance	Pribyl et al. 2005
South Platte River, CO	Mixed	4820	286	Whole stream MIMS	Pribyl et al. 2005
South Platte River, CO	Mixed			Mass balance	Sjodin et al. 1997
	upstream	131 – 7290	188 – 675		
	downstream	507 – 2560	148 – 358		

[1]AIT = acetylene inhibition technique
[2]AIT + C = acetylene inhibition technique with chloramphenicol added to suppress new enzyme synthesis
[3]MIMS = membrane inlet mass spectrometry
[4]IRMS = isotope ratio mass spectrometry

In this study, denitrification was greater in the agricultural stream compared to the silvicultural stream. Among literature values, denitrification rates were generally lowest in forested streams compared to watersheds supporting agricultural and suburban land uses (Table 3.3). In a comparison of 9 headwater streams in three land uses (forest, agriculture and suburban), Inwood and others (2005) measured the highest inorganic nutrient and DOC concentrations in the agricultural streams compared to the lowest in the forested streams. These same trends also were reflected in denitrification rates which were most closely correlated with NO_3-N concentrations and secondarily controlled by DOC, O_2, temperature and sediment organic matter.

Comparison of denitrification rates in this study as a function of stream order showed a decline along the flowpath from the 1st order headwater streams to the 4th order estuarine creeks in both land uses. These spatial trends in denitrification were most closely correlated with sediment organic C. High levels of organic matter in the 1st order ditches enhanced biological activity by providing both a C source and source of NO_3-N via mineralization. Whereas high streamflow during storm events caused significant scouring and reworking of bed sediments at downstream sites exposing sandy sediments and possibly displacing denitrifying communities downstream.

3.4.1 Biochemical controls

Denitrifiers use NO_3-N as an electron acceptor in the decomposition of organic matter when O_2 concentrations become limiting. As such, NO_3-N, O_2 and organic C are expected to exert considerable control on rates of denitrification. The influence of these environmental conditions was investigated through enrichment experiments and correlations with in situ rate measurements.

Concentrations of NO_3-N in both streams in this study were consistently low during baseflow suggesting denitrification was potentially coupled with nitrification. Significant correlations were observed between denitrification and sediment O_2 demand, which is a measure of total community metabolism and includes heterotrophic respiration as well as other O_2 consumptive processes such as nitrification. This relationship suggests control of denitrification by the availability of electron donors (organic C) and/or nitrification linked to organic N mineralization rates. A linear relationship between denitrification and O_2 flux has been reported elsewhere in environments influenced by coupled nitrification-denitrification (Seitzinger 1994, Seitzinger and Giblin 1996), particularly those with low surface water NO_3-N concentrations (Cornwell et al. 1999, Kemp and Dodds 2002a). Although low NO_3-N concentrations during baseflow and positive correlations with sediment O_2 demand do not provide direct evidence, they suggest that nitrification may be an important N cycling process in both streams.

During storm events, pulses of NO_3-N were observed in the agriculturally influenced streams. As such, the potential for increased removal of stream water NO_3-N via denitrification was investigated in a series of enrichment experiments. Experiments conducted in conjunction with increased temperatures and elevated DOC all showed a consistent stimulatory impact of NO_3-N on denitrification rates. Additionally, the combination of increased temperature and elevated NO_3-N resulted in highest rates. Other studies have shown similar NO_3-N control of denitrification in low NO_3-N streams (Holmes et al. 1996, Martin et al. 2001) as well as NO_3-N rich streams (Pinay et al. 1993, Garcia-Ruiz et al. 1998a, Royer et al. 2004, Smith et al. 2006). Similar to observations in other studies, temperature was a secondarily controlling factor on denitrification rates in the agricultural stream similar (Christensen et al. 1990, Pinay et al. 1993, Pattinson et al. 1998).

Correlations between two indicators of organic C (sediment organic C and water column O_2 flux) and denitrification indicated potential C limitation. However, enrichment experiments in both watersheds showed no response to additions of labile DOC alone (but see Chapter 3). Under conditions in which coupled nitrification-denitrification is an important N pathway, labile organic C may actually hinder denitrification by providing a better quality substrate for heterotrophic metabolism thereby increasing competition for NH_4-N and reducing nitrification rates (Strauss and Lamberti 2002).

The lack of any response to increased NO_3-N, C and temperature in the silvicultural stream was unexpected and could indicate that denitrifying communities were present but at low population densities. Alternatively, the pH of the stream may have inhibited denitrification rates. Slightly acidic conditions were measured in the silviculture stream (5.5 – 6.5) compared to a neutral pH consistently measured in the agriculture stream. The optimum pH for denitrifiers is 7.0 – 8.0. At lower pH values, denitrification rates decrease and the proportion of N_2O to N_2 increases (Knowles 1982).

3.4.2 Hydrologic control

Since nitrate is the most mobile form of N, its removal is important to downstream water quality especially during storm events when NO_3-N transport is high. Low retention times during high flow often result in increased downstream transport (Bohlke et al. 2004, Gucker and Pusch 2006). Martin and others (2001) investigated the potential for denitrification to remove instream NO_3-N in forested streams. Although the authors identified NO_3-N as the primary factor controlling denitrification, it was a small sink and accounted for <2% of stream N export in both systems. In a study conducted by Royer et al. (2004), considerably higher denitrification rates were measured in agricultural stream sediments, but due to high instream N concentrations and high flow rates during storm events, loss rates attributed to denitrification were <5% per day.

Previous studies have shown that instream denitrification may become saturated at high concentrations (Kemp and Dodds 2002a, Bernot and Dodds 2005). Biotic control of denitrification occurs most frequently in streams with chronically high NO_3-N concentrations and is subject to saturation as described by Michaelis-Menton kinetics. Conversely, in streams with periodically high NO_3-N (e.g. storm pulses), denitrification may be more influenced by hydrodynamic limitations of mass transport and denitrification rates would be expected to increase in proportion to increased NO_3-N concentration (Dodds 2002). Results of an experiment in the agricultural stream in which denitrification increased linearly with progressively increasing NO_3-N concentrations indicated that denitrification was not saturated and thereby likely controlled by hydrological transport of water column NO_3-N to zones of denitrification in the sediments. These streams have the potential for high rates of NO_3-N removal via denitrification, but streamwater NO_3-N must first be transported to these sediment denitrifying communities.

These trends were common among many studies in a wide range of ecosystems with varying topography, climate and land use (Jansson et al. 1994, Williams et al. 2004, Inwood et al. 2005). Although high rates were measured in some cases, total contributions to downstream export of NO_3-N were negligible. These results are best explained by hydrologic controls on NO_3-N retention. In order for denitrification to have a significant impact on instream concentrations, streamwater must first be transported to denitrifying sediment communities. Scaling up denitrification rates which are typically measured during baseflow conditions based on surface area of the streambed neglects this fundamental link. Without evidence or mechanisms for surface water exchange, denitrification may have a limited impact on water quality.

3.4.3 Loss rates and mass balance of nitrate

Mass transfer velocity is a measure of biologic nutrient uptake independent of hydrologic characteristics and nutrient concentrations (Doyle 2005). Denitrification rates measured in the agricultural stream in this study were expressed as NO_3-N flux and converted to $V_{f,den}$ based on instream NO_3-N concentration. Results from this study suggest that denitrification may have been fueled by nitrification when concentrations were low during baseflow and by streamflow NO_3-N when concentrations were elevated surrounding storm events. Additionally, NO_3-N stimulated denitrification in these sediments and was positively correlated with NO_3-N under enriched conditions. As such, conversion of laboratory based measurements to NO_3-N flux and ultimately to reach retention metrics provided a method for assessing the potential for denitrification to remove instream NO_3-N.

Denitrification mass transfer velocities were within the range reported for reach-scale V_f at other sites (Ensign and Doyle 2006). Although denitrification is only a component of NO_3-N uptake, similarity between $V_{f,den}$ and reach-scale V_f suggests that denitrification may play an important role in reducing NO_3-N transport.

Values of -*k* were considerably higher than those published for other headwater streams with loss rates at most sites >100% per day (Alexander et al. 2000, Royer et al. 2004). Loss rates exceeding 100% d^{-1} indicate that the demand by instream denitrification could potentially remove 100% of the NO_3-N load entering the stream in the absence of nitrification or other NO_3-N sources. High estimated loss rates for these sites were likely the result of low instream concentrations, shallow water depth and long residence times during baseflow conditions. Additionally, these loss rates were calculated from laboratory rate measurements and do not take into account the effects of stream hydrology.

A NO_3-N mass balance was calculated for this reach using instream monitoring data from upstream and downstream stations (see Chapter 2). Nitrate concentrations increased along the reach with the mass balance resulting in a net negative retention. The disparity between high loss rates attributed to denitrification and reach scale gains of NO_3-N is potentially due to nitrification in oxidized streambed sediments releasing NO_3-N to the water column and/or inputs of high NO_3-N groundwater. In a similar agricultural watershed in the upper Mississippi River basin, Bohlke and others (2004) quantified instream NO_3-N retention and denitrification by using ^{15}N-NO_3 as an instream tracer. Researchers measured denitrification rates similar to those measured in core incubations in this study (120 ± 20 μmol m^{-2} h^{-1}). Despite this removal, the reach was gaining NO_3-N due to nitrification and groundwater inputs.

3.5 Conclusions

Denitrification is one component of instream NO_3-N retention that is particularly important because it removes N from the aquatic ecosystem and has been identified as the primary mechanism for NO_3-N retention in headwater streams (Seitzinger 1988, Alexander et al. 2000). Other biological and physical processes, including assimilation, burial and reduction to NH_4-N, allow NO_3-N to remain within the potential reactive N reservoir. High denitrification rates have been measured in a variety of ecosystems and climates but its contribution to nitrate retention appears low in some cases (Williams et al. 2004, Inwood et al. 2005, Smith et al. 2006). These trends are most likely explained by hydrologic drivers: high flow rates and decreased retention time associated with large pulses of NO_3-N during storm events. These conditions provide little opportunity for denitrification to remove instream NO_3-N.

In the coastal streams in this study, denitrification appeared to be most closely dependent on NO_3-N supply. The potential exists for denitrification rates to respond in a linear fashion to increasing NO_3-N concentrations. This is particularly important in coastal watersheds where nutrient concentrations and discharge are intrinsically linked. The ability of denitrification to attenuate storm pulses of NO_3-N depends largely on hydrological transport of nitrate-rich stormflow to denitrifying communities in streambed sediments. Management of these drainage networks including channel modifications to increase hyporheic flow (i.e. addition of woody debris or other channel obstructions) or increase retention time (i.e. flashboard risers or streamside wetlands) may help reduce downstream export in streams that support high rates of denitrification.

CHAPTER 4:

INFLUENCE OF SENESCENT ALGAL BLOOMS ON DENITRIFICATION IN AGRICULTURAL HEADWATER STREAMS

4.1 Introduction

Headwater streams have been shown to play a critical role in nutrient retention and may help control nitrogen (N) export to downstream receiving waters (Alexander et al. 2000, Peterson et al. 2001). High biological activity in these environments is attributed to close connection with the landscape creating hot spots where nutrients and carbon (C) supplies are both maximized. Nitrogen transformations are particularly important in coastal regions where excessive N loads have contributed to increased frequency of algal blooms, eutrophication, habitat degradation and reduced biodiversity (Nixon 1995, Boesch et al. 2001).

The primary mechanisms for N retention and removal in streams include algal assimilation, heterotrophic uptake and denitrification. Assimilatory uptake by vascular plants, algae, and microbes generally represents only short-term retention of N because the organic N is eventually remineralized. Of these processes affecting instream NO_3-N retention, denitrification is the only process that results in a net loss of N from the system (Galloway et al. 2003, Seitzinger et al. 2006).

Recent reach-scale uptake experiments have shown the importance of algal uptake, particularly in the retention of instream ammonium (NH_4-N) (Mulholland et al. 2000, Hamilton et al. 2001). In agricultural watersheds, fertilizer application leads to high instream nutrient concentrations, especially following storm events. In addition, native riparian vegetation is often removed to allow planting of crops up to the water's edge which reduces the canopy and allows higher amounts of light to reach the water surface. This combination enhances the potential for algal blooms, particularly during the spring and summer. While algal assimilation of instream nutrients may be beneficial by reducing pulses of nutrient export, this retention is only temporary. As algal biomass senesces, both organic and inorganic forms of N, phosphorus (P) and C are released back to the stream. This slow release attenuates the pulse observed during storm events, but ultimately these stored nutrients are returned to the stream ecosystem. The primary goal of this study was to assess the fate of this "re-released" N and quantify the proportion of it that is denitrified. This study tested the hypothesis that uptake of instream N by algae and subsequent N release from senescent algal biomass enhances denitrification in coastal agricultural watersheds.

4.2 Methods

4.2.1 Site description

This study was conducted in first order streams in a small (7.7 km^2) agricultural watershed in Eastern North Carolina that drains into the South River via Southwest Creek, and subsequently the Neuse River Estuary (Figure 4.1).

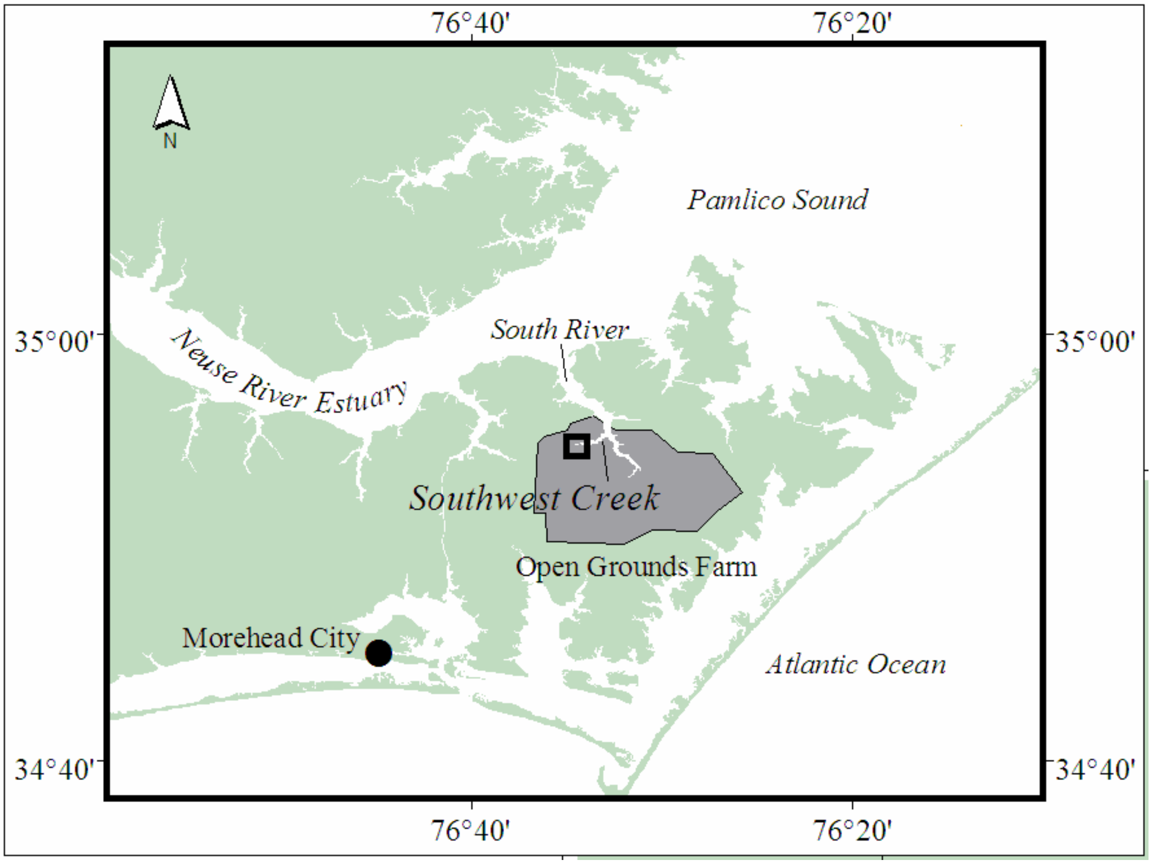

Figure 4.1. Location of the study area in eastern North Carolina, USA.

The watershed is located on Open Grounds Farm (OGF), a 182 km^2 row-crop farm with annual crop rotation between corn and soybeans. OGF utilizes best management practices (BMPs), including no-till planting to reduce loss of sediment and variable rate fertilizer application according to detailed soil maps developed annually. First order drainage ditches traverse the fields at 100 m intervals and connect with second order canals to make up the drainage network. These engineered ditches and canals are entrenched 1 m and 3 m deep, respectively and have homogenous bed sediments and limited riparian vegetation due to annual clearing and dredging activities. Canals are equipped with flashboard risers managed to facilitate drainage following frequent spring storms during planting and to raise the water table during dry periods in the summer growing season.

Although there is limited riparian vegetation, the absence of tile drainage commonly found in agricultural watersheds in the Midwestern United States allows for nutrient attenuation along this flow path. Best management practices combined with the lack of tile drainage contribute to average instream concentrations lower than those observed draining agricultural watersheds in the Midwestern U.S. (Royer et al. 2006).

Ample sunlight due to limited riparian vegetation, warming temperatures and high nutrient concentrations contribute to high algal biomass in the streams, particularly during the spring following fertilizer application. Algae in the study streams were primarily two common green algal species, *Mougeotia sp*. and *Hydrodictyon sp*., common in eutrophic lakes and rivers. Both species initially colonize sediment and plant surfaces. As their productivity and biomass increase, mats of these filamentous algae become buoyant due to the formation of oxygen bubbles in the mats matrix.

4.2.2 Water chemistry

Water samples were filtered through Whatman GF/F glass fiber filters (25mm diameter, 0.7 μm nominal pore size) and the filtrate was analyzed with a Lachat Quick-Chem 8000 automated ion analyzer for NO_3-N, NH_4-N, PO_4-P and total nitrogen (TN) concentrations using standard protocols (Lachat Instruments, Milwaukee, WI, USA: NO_2/NO_3 Method 31-107-04-1-A, NH_4 Method 31-107-06-1-A, PO_4-P Method 31-115-01-3-G, and TN Method 31-107-04-3-B). Dissolved organic nitrogen (DON) concentrations were calculated as the difference between TN and dissolved inorganic nitrogen (NO_3-N + NH_4-N) concentrations. Dissolved organic carbon concentrations were determined using a high temperature combustion technique on a Shimadzu model TOC-500, equipped with an ASI-5000A autosampler. Particulate nitrogen (PN) in the water column and of the algal biomass was determined by carbon, hydrogen, nitrogen analysis (CHN). For the water samples, 25 ml water was filtered on precombusted (500 ºC) GF/F filters. Subsamples of the algal biomass were taken in triplicate and analyzed to determine the mass of C and N per dry weight. Filters and algal biomass samples were dried for 24 h at 60°C, fumed for 8 h with 12N HCl to remove inorganic C, and re-dried. Fumed samples were analyzed for organic C and N content with a Perkin Elmer CHN analyzer (Model 2400 Series II) standardized with acetanilide.

4.2.3 Nutrient loads

Flow and water quality parameters were monitored at 30-minute intervals at the outlet of the watershed from August 2003 through July 2006. An area-velocity flow meter was mounted in the outlet end of a drainage pipe and linked to an automated ISCO water sampler and data recorder (Model 6712, ISCO, Lincoln, NE). Stream water samples were collected weekly for analysis of NH_4-N, NO_3-N, and PO_4-P. In addition, more frequent automated flow-paced sampling was conducted during storm events. A continuous record of nutrient concentrations was developed by interpolating between observed concentrations.

Streamflow was separated into baseflow and storm components using a graphical baseflow separation method that accounted for watershed size (Dingman 1994). Nutrient loads were determined by multiplying interpolated nutrient concentrations by measured 30-minute flow rates. Hurricane Isabel made landfall during September 2003 and Hurricane Ophelia during September 2005. These storms necessitated the evacuation of instrumentation from the field site. No data were available surrounding these events due to high flow conditions that persisted for several weeks and prevented immediate redeployment of instruments.

4.2.4 Denitrification rates

Experiments were conducted during the spring in 2004 and 2005 to quantify the influence of storm-derived N and senescing algal mats on denitrification rates. Nitrate addition experiments were conducted in April 2004 and 2005; the algal leachate addition experiment was conducted in May 2005.

The influence of storm-driven nutrient pulses on rates of denitrification in stream bed sediments was simulated by measuring denitrification in sediment cores with overlying water amended to 100 μM sodium nitrate ($NaNO_3$). Sediment cores (6 cm diameter x 20 cm depth) were collected from the 1st order ditches in triplicate in clear PVC tubes and returned to the laboratory for analysis. In addition, one water blank was included to account for activity in the water column and any methodological errors that might be present. Cores were incubated in water collected from the site amended with $NaNO_3$ and held overnight at *in situ* temperature for approximately 18 hours. At the start of the experiment, cores were capped with gas tight tops equipped with sampling ports and a suspended magnetic stirbar to keep the water column well-mixed while not disturbing the sediment surface. During 2004, the NO_3-N additions were performed on 3 replicate cores and compared to one water blank. In 2005, the experiment was conducted with 6 replicate cores and 2 water blanks.

Time series sampling of overlying water was conducted for analysis of dissolved gases (N_2, O_2 and Ar) by membrane inlet mass spectrometry (MIMS) (Kana et al. 1994).

Denitrification was operationally defined as the net positive flux of N_2 out of the sediment. By using argon as a conservative tracer, subtle changes in the ratio of N_2:Ar in overlying water can be measured at high precision (0.05%). Sediment rates were corrected for water column processes and other potential methodological errors by subtracting net N_2 fluxes measured in corresponding water blanks. Experiments were conducted over short time intervals (typically 4-6 hours) to prevent depletion of dissolved oxygen to levels less than 75% of initial concentrations.

To assess the impact of senescing algae (identified as *Mougeotia sp*. and *Hydrodictyon sp.*) on denitrification, an algal leachate addition experiment was conducted during May 2005. Algal biomass was collected from the first order stream, returned to the lab and freeze dried. The biomass was combined and mixed to form a homogeneous mixture. On the first day of the experiment, 200 L of stream water was collected and separated into 4 – 50 L containers. These were placed in an outdoor experimental pond to maintain ambient temperature and allow sunlight to reach the water surface. Freeze-dried algal biomass (25 g per container) was added to each container over several days to create an algal leachate and simulate various stages of algal senescence. Table 4.1 shows the four treatments, relative ages of algal leachate and initial nutrient concentrations.

Table 4.1. Nitrogen and carbon concentrations for each treatment during the algal leachate experiment conducted in May 2005.

Treatment	Leachate age (h)	NO_3-N (uM)	NH_4-N (uM)	DON (uM)	PON (mg/L)	DOC (mg/L)	POC (mg/L)
Control	0	16.0	1.1	113.6	0.25	37.9	2.08
A	6	25.2	12.0	185.5	0.63	43.0	4.62
B	17	24.1	14.2	183.7	1.60	48.6	6.66
C	64	14.6	31.4	236.9	1.25	45.6	7.43

On the last day of algal leaching, sediment cores were collected from the 1st order stream and returned to the lab. Stream water in each core chamber was replaced with algal leachate from each of the 4 treatments immediately before initiating the experiment. Denitrification rates were then measured using the MIMS method described above.

Concentrations of NH_4-N, NO_3-N, total dissolved N (TN) and dissolved organic C (DOC) were measured at the beginning and end of each experiment. Nutrient fluxes were calculated based on the change in concentration over the incubation period. Oxygen flux was measured during all experiments via MIMS and defined as the change in dissolved O_2 concentration in the water column over time. Oxygen fluxes were also measured in water blanks. The rate of depletion of O_2 in water samples can be used as a method for indirectly measuring the quantity of readily biodegradable organic matter. Biochemical oxygen demand (BOD) tests are often employed in wastewater treatment as a relatively inexpensive and efficient way of measuring biologically available organic matter concentrations in surface water (Metcalf and Eddy Inc. 1991). The rate of consumption depends largely on the quality of the organic matter, the bacterial communities present and temperature. Since all treatments were taken from the same stream, it can be assumed that bacterial communities were similar among all treatments. In addition, the temperature was maintained at 18 ºC for all treatments. Therefore, the rate of O_2 consumption in the water blanks can be used to estimate the quality of the organic C.

4.2.5 Calculations and statistics

The additional N supplied by remineralization of the algae in each treatment was estimated as the concentration difference between the control (no algal leachate added) and each treatment. Additional dissolved N (as TN and NO_3-N) was estimated using concentrations at the start of the experiment in each treatment compared to the control:

$$N\ supplied\ by\ algae\ (\mu M) = N_{treatment}\ (\mu M) - N_{control}\ (\mu M) \qquad (4.1)$$

Denitrification rates from each treatment were used to quantify the mass of N denitrified during the 3 hour experiment:

$$N\ denitrified\ (\mu mol) = Den\ (\mu mol\ m^{-2}\ h^{-1})\ x\ area\ (m^{2})\ x\ duration\ (h) \qquad (4.2)$$

This number was divided by the total mass of N supplied by the algae to determine the percent of algal N denitrified.

Linear regressions were performed to relate denitrification rates to chemical characteristics of the water column (dissolved and particulate nutrient and carbon concentrations) and fluxes of relevant constituents (including nutrients, carbon and oxygen). Significant relationships were determined using a one-way ANOVA with post-hoc comparison of means using Tukey's Honest Significant Difference. All statistical analyses were conducted using R statistical computing software R Development Core Team 2007.

4.3 Results

4.3.1 Instream water quality

Measurements of instream water quality during baseflow and storm events showed a significant impact of stormwater runoff on instream nutrient and carbon concentrations. Pulses of NO_3-N from the watershed during storms consistently resulted in nitrate concentrations higher than those observed during baseflow (Figure 4.2). Median NO_3-N concentrations during baseflow conditions were 7.0 μM and 54.1 μM during storm events. However, no difference was observed between baseflow and storm events for both DOC and NH_4-N. These data also reveal that NO_3-N was the dominant form of dissolved inorganic nitrogen (DIN) particularly during storm events.

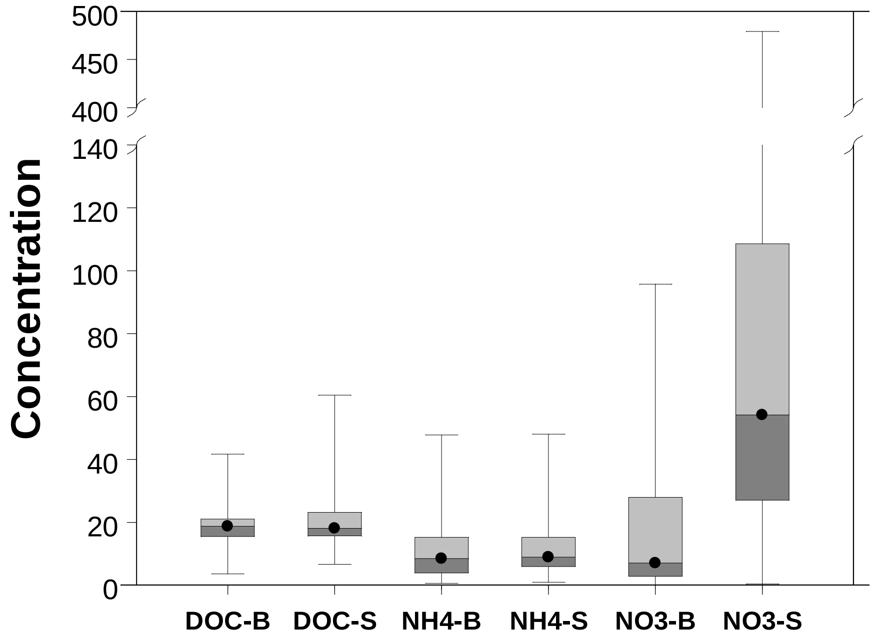

Figure 4.2. Instream concentrations during baseflow (B) and storm events (S). The middle line represents the median of the data, upper and lower box extents are the 25th and 75th percentiles respectively and the whiskers are the minimum and maximum observed values. Concentrations are shown in mg/L for DOC and μmol/L for NH_4-N and NO_3-N.

4.3.2 Denitrification rates after NO_3-N addition

Denitrification rates in sediments collected from 1st order ditches were measured following the addition of $NaNO_3$ on two occasions, April 2004 and 2005. The goal was to obtain target concentrations of NO_3-N during both experiments similar to those observed during storm events (Figure 4.2). For the 4/2004 experiment, the concentrations of NO_3-N in the control and N addition were 0.3 uM and 80 uM respectively. In 2005, the concentrations of NO_3-N were 0.2 uM in the control and 96.4 uM in the N addition. Both experiments were conducted during a baseflow period when ambient instream N concentrations were consistently low in order to compare the effects of elevated N observed during storm events.

Denitrification rates measured in N-amended sediment cores were significantly higher during both experiments (Figure 4.3). One-way ANOVA with Tukey's post-hoc comparison of means was used to compare differences between the treatments and to test the robustness of these differences ($p < 0.001$ for both 2004 and 2005 experiments).

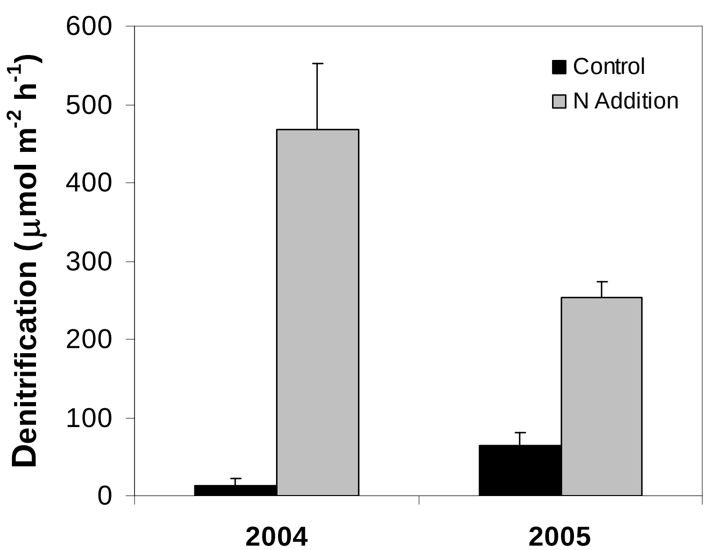

Figure 4.3. Denitrification in sediment cores showing increased rates upon addition of $NaNO_3$. Error bars indicate one standard error.

Denitrification rates were significantly correlated with both instream NO_3-N concentrations (2004: $R^2 = 0.878$, $p < 0.01$; 2005: $R^2 = 0.839$, $p < 0.0001$) and net NO_3-N flux into the sediments (2004: $R^2 = 0.985$, $p < 0.001$; 2005: $R^2 = 0.756$, $p < 0.001$).

4.3.3 *Algal leachate addition experiment*

The influence of senescing algal blooms on rates of denitrification in streambed sediments was measured by amending sediment cores with algal leachate during May 2005. Concentrations of nutrients and C (both dissolved and particulate forms) were measured throughout the 3 day experiment (Table 4.1). Dissolved inorganic N and DON increased quickly during the first 6 hours (Treatment A) but remained at that level 11 hours later (Treatment B). In contrast, PON continued to increase through the first 17 hours. The algal biomass in Treatment C was allowed to leach for nearly 3 days. At this point, NH_4-N and DON concentrations continued to increase while NO_3-N and PON concentrations decreased.

Similarly, concentrations of POC and DOC increased quickly during the first 17 hours with DOC increasing from 37.9 mg/L to 48.6 mg/L and POC increasing from 2.08 mg/L to 6.66 mg/L. After nearly 3 days of leaching (Treatment C), DOC concentrations decreased slightly while POC concentrations continued to increase.

Carbon to nitrogen ratios of dissolved and suspended particulate material were calculated for each core at the end of the experiment (Figure 4.4). The ratio of DOC to TN was used for the dissolved components. CHN analysis of the filtered particulate was used to calculate C:N ratios for the suspended particulate fraction. Comparisons of particulate C:N ratios revealed that the highest ratios were measured in the Control and Treatment A (C:N ratios of 9.0 and 9.5 respectively). The greatest dissolved C:N ratio was also in the Control. Treatment B was observed to have the lowest particulate C:N ratio of 5.5, while Treatment C had the lowest dissolved C:N ratio of 13.4.

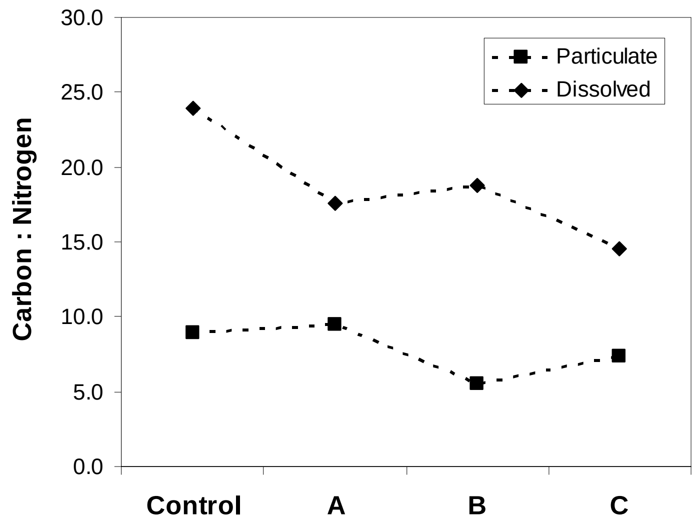

Figure 4.4. Suspended particulate and dissolved C:N ratios in the water column for each sediment core treatment.

Denitrification rates exhibited a significant stimulatory response from the addition of the algal leachate, with treatments B and C being 3 and 2 times greater than the control respectively (Figure 4.5). Although all treatments showed a trend towards higher rates of denitrification upon addition of the algal leachate, only Treatment B was statistically different from the control ($p < 0.05$).

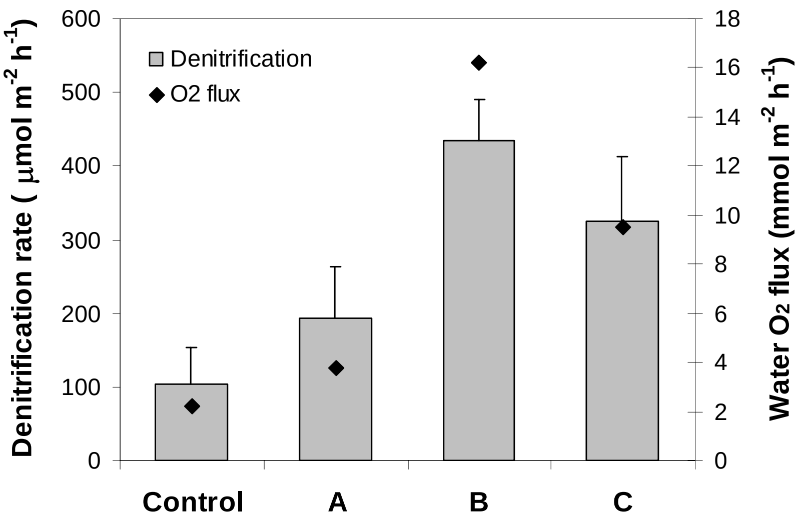

Figure 4.5. Denitrification and water blank oxygen flux after addition of algal leachate (see Table 1 for leachate age and water chemistry of each treatment). Error bars are one standard error.

Oxygen flux in the water blank associated with each treatment was used as an estimate of the quality of dissolved carbon (Figure 4.5). Treatments B and C had much greater oxygen consumption (16.2 and 9.5 μmol m^{-2} h^{-1}) compared to the control (2.2 μmol m^{-2} h^{-1}) and Treatment A (3.8 μmol m^{-2} h^{-1}).

A linear regression analysis was performed between denitrification rates and various chemical characteristics of the overlying water and fluxes of relevant constituents (Table 4.2). While there was no correlation between NO_3-N concentrations in the water column and rates of denitrification, there was a significant correlation between NO_3-N flux into the sediment and denitrification ($p < 0.05$). The strongest correlations were observed between denitrification and measures of C quantity (measured as concentration; DOC: $p < 0.05$, POC: $p < 0.01$ and PON: $p < 0.01$) and C quality (water O_2 flux: $p < 0.01$ and C:N ratio: $p < 0.01$).

Table 4.2. Linear regressions between rates of denitrification (y) and chemical characteristics of the water column and fluxes of relevant constituents (x). Sample size was 12 in all correlations. Significant p values are denoted with "*" indicating significance at the 95% confidence level and "**"at the 99% confidence level.

Factor	Equation	R^2	p
NO_3	y = 7.174x + 120.977	0.046	0.503
NH_4	y = 7.001x + 161.523	0.233	0.112
DOC	y = 30.979x - 1091.889	0.603	0.003**
POC	y = 53.667x - 14.656	0.501	0.010**
PON	y = 238.663x + 41.670	0.633	0.002**
DOC:TN	y = -22.005x + 676.104	0.231	0.113
C:N	y = -73.2405x + 836.987	0.570	0.005**
Sediment NO_3 flux	y = 0.669x + 79.309	0.338	0.047*
Sediment NH_4 flux	y = -0.236x + 191.561	0.066	0.419
Sediment DOC flux	y = 4.277x + 146.507	0.206	0.138
Water O_2 flux	y = 22.312x + 87.536	0.611	0.003**
Sediment O_2 flux	y = -21.302x + 348.255	0.125	0.259

Comparisons of denitrification rates to NO_3-N and NH_4-N flux measurements showed NO_3-N fluxes consistent with denitrification fueled by water column NO_3-N (Figure 4.6). In sediment cores amended with algae leachate (Treatments A, B and C), denitrification and NO_3-N fluxes were nearly equal with high negative NH_4-N fluxes (out of the sediments).

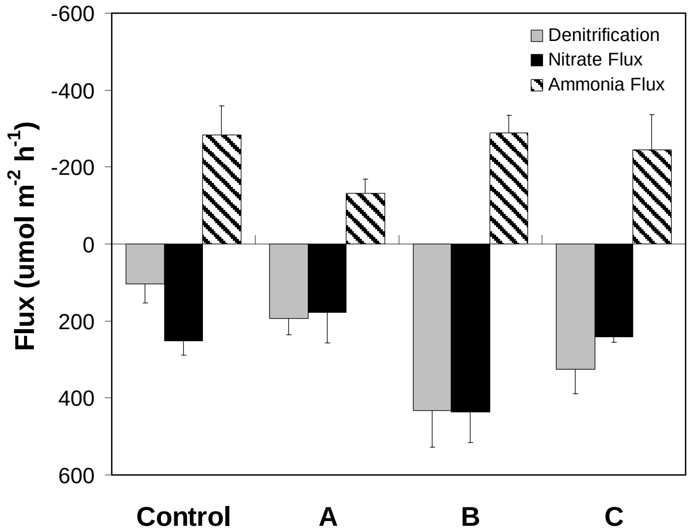

Figure 4.6. Rates of denitrification and N fluxes after addition of algal leachate (see Table 1 for leachate age and water chemistry of each treatment). Positive flux measurements indicate movement from the water column into the sediment. Error bars are one standard error.

Denitrification rates in amended cores during the short duration (3 h) experiment ranged from 193.8 to 325.2 μmol m^{-2} h^{-1} (Table 4.3). Additional NO_3-N and TN present in overlying water due to remineralization of the algal biomass ranged from 7.1 to 8.8 μM and 86.3 to 151.1 μM respectively. Denitrification removed 34.6 – 100% of additional NO_3-N and 3.2 – 8.1% of the additional TN supplied by senescing algal biomass.

Table 4.3. Concentration of NO_3-N and TN supplied by senescing algae and the proportion of the additional N that was denitrified during the 3 hour experiment. Uncertainty is one standard error.

Treatment	Denitrification (μmol m^{-2} h^{-1})	Additional NO_3-N (μM)	Algal NO_3-N denitrified (%)	Additional TN (μM)	Algal TN denitrified (%)
Control	104.6 ± 48.9	--	--	--	--
A	193.8 ± 68.8	8.8 ± 0.5	34.6 ± 13.7	86.3 ± 4.4	3.5 ± 1.4
B	433.6 ± 55.9	7.1 ± 0.9	100.0 ± 20.3	86.4 ± 7.5	8.1 ± 1.4
C	325.2 ± 88.0	-1.5 ±0.5	0.0	151.1 ± 4.3	3.2 ± 0.7

4.4 Discussion

Headwater steams have consistently been identified as critical areas for retention of nutrients, particularly inorganic N. Numerous biogeochemical processes influence the concentrations during transport through stream networks, resulting in a net decrease of nutrients as water moves downstream (Alexander et al. 2000, Peterson et al. 2001). It was estimated that 60% of the N entering a watershed's stream network may be retained (permanently via denitrification or temporarily through biotic sequestration) within streams in the northeastern United States (Seitzinger et al. 2002). Alteration of nutrient concentrations by riverine processes during transport also changes the timing of nutrient delivery and the quality of nutrients exported to downstream ecosystems (Cooper and Cooke 1984). Assimilatory uptake of nutrient pulses during storm flow reduces peak concentrations while remineralization returns both inorganic and organic forms to the stream during low flow conditions.

In this agriculturally influenced stream, instream concentrations of NO_3-N and mass export of N were primarily controlled by precipitation. A concentration pattern was observed in instream NO_3-N concentrations similar to results reported elsewhere (Ocampo et al. 2006, Poor and McDonnell 2007). The concentration pattern is characterized by an observed increase in NO_3-N with increased stream discharge that essentially mimics the hydrograph. In contrast, remineralization of organic matter during low flow conditions resulted in slightly higher concentrations of NH_4-N and DOC during baseflow. Increased flow during storm events diluted instream concentrations (Ensign et al. 2006).

4.4.1 Controls on denitrification

Denitrification is performed by facultative heterotrophic bacteria during the decomposition of organic matter. They use O_2 as the electron acceptor in aerobic environments, but are capable of switching to NO_3-N in low-oxygen conditions. The major end product is gaseous N_2 with lesser production of N_2O and NO, all of which are released to the atmosphere, constituting a removal of N from the system (Knowles 1982). In systems with excess N, denitrification is a desirable process because it reduces transport to aquatic ecosystems where large inputs of N can lead to excessive productivity of aquatic plants and algae. Bacteria capable of denitrification are ubiquitous allowing denitrification to occur in a wide range of terrestrial and aquatic environments provided the following conditions are met: low O_2 concentrations (< 0.2 mg/L), NO_3-N availability and sufficient quantity and quality of organic C. High variability among denitrification rates has been observed with the range of N loss in headwater streams spanning two orders of magnitude (Jones and Holmes 1996, Seitzinger et al. 2006).

Provided reduced conditions occur, NO_3-N availability should be a strong predictor of denitrification, particularly when NO_3-N concentrations are low (Martin et al. 2001, Kemp and Dodds 2002b). Nitrate concentrations in the unamended controls in these experiments representing baseflow conditions were low (0.2 – 16 μM) compared to other agricultural streams (Kellman 2004, Royer et al. 2004). Denitrification measured in sediment cores amended with NO_3-N showed significantly higher rates than unamended controls indicating supply of NO_3-N strongly influenced rates of denitrification.

In nitrate-rich streams, denitrification tends to be supported by instream sources of NO_3-N as opposed to coupled nitrification-denitrification (Christensen et al. 1990). Utilization of NO_3-N from the water column is particularly relevant in agricultural streams experiencing NO_3-N pulses during storm events. Despite high denitrification rates, studies of similar agricultural streams in Illinois, USA (Royer et al. 2004) and Sweden (Jansson et al. 1994) found that sediment denitrification had minimal impact on N export. The proportion of N inputs removed by denitrification in a stream reach is typically quite small (1-20%), while the cumulative effect of continued N removal via denitrification along the entire flow path through the riverine system can be as much as 30-70% of total N inputs (Galloway et al. 2003).

4.4.2 Interactions between denitrifiers and instream algae

In addition to denitrification, several instream processes have been identified as critical retention mechanisms of watershed derived N, including uptake and assimilation by algae, aquatic plants and heterotrophic bacteria and sedimentation of particulate N. From a nutrient management perspective, denitrification is particularly desirable because it removes inorganic N from the stream, whereas other assimilative processes transform it into organic forms which will eventually be remineralized and released downstream.

In this agriculturally influenced stream, algae attached to sediment and plant surfaces proliferated during low flow conditions. Many interactions can occur between these communities and denitrifying bacteria, some enhancing rates of denitrification and others reducing it. Photosynthesic benthic algae significantly impact O_2 penetration into surface sediments (Christensen et al. 1990, Nielsen et al. 1990, An and Joye 2001). By increasing O_2 concentrations in surface sediments, algae stimulate nitrification rates and indirectly increase the supply of NO_3-N to denitrifiers. In shallow, sub-tidal estuarine sediments, O_2 production by benthic microalgae enhanced rates of coupled nitrification-denitrification which resulted in significant loss of NO_3-N from the sediments (An and Joye 2001).

The resultant increased thickness of the oxidized sediment layer also pushes the NO_3-N reduction zone deeper into the sediment. This increases the transport distance for NO_3-N from the water column to zones of denitrification thereby potentially reducing removal of NO_3-N from the stream. In a nitrate-rich Danish lowland stream, NO_3-N from the water column was shown to be the source of NO_3-N for denitrifiers. Photosynthetic O_2 production increased the oxic zone and decreased denitrification activity up to 85% during the spring (Christensen et al. 1990).

The diel cycle of photosynthesis in these algal communities results in peak O_2 production during the day and consumption during the night. This has been shown to lead to different processes dominating sediment biogeochemistry at different times (Christensen et al. 1990, Nielsen et al. 1990). During the day, algae are more successful at incorporating available inorganic nutrients compared to bacteria (both heterotrophic and denitrifying) resulting in relatively low rates of denitrification during this time. At night, photosynthesis ceases and O_2 is rapidly consumed within the sediments allowing high rates of denitrification to take place.

4.4.3 Instream nutrient retention by algal uptake and denitrification

Buoyant mats of filamentous algae were frequently observed in the agricultural streams during spring and summer following fertilizer addition. Although algal biomass was not directly measured as a part of this study, the mats were often observed to cover a large proportion of the water surface. Comparisons of sediment denitrification to denitrification in biofilms on plant surfaces and in algal mats have shown that plant-associated biofilms had a substantially lower areal denitrification rates (Kemp and Dodds 2002b, Schaller et al. 2004). These results suggest the algal mats may play a relatively minor role in N removal via denitrification. However, the impact of remineralized nutrients and labile organic matter from senescing algae on denitrification rates has not yet been established.

A source of natural, bioavailable dissolved organic carbon was created by amending stream water with algal biomass and allowing it to leach for several hours to days. This leachate showed significant differences in C and nutrient concentrations compared to unamended stream water. Nitrogen concentrations (DON, PON, NO_3-N and NH_4-N) increased rapidly during the first 17 hours of leaching in the stream water. These changes were likely attributed to decomposition of algal biomass to particulate and dissolved forms of organic N, further bacterial remineralization and photodegradation to inorganic N (primarily as NH_4-N) and nitrification of NH_4-N to NO_3-N. After nearly 3 days of leaching, NH_4-N concentrations continued to increase while NO_3-N decreased, which could indicate a slowing of nitrification rates as more labile carbon fractions were utilized. Studies have shown that nitrifiers are generally less competitive for NH_4-N compared to heterotrophic bacteria when organic carbon quality decreases (Strauss and Lamberti 2002).

Increased denitrification rates amended with algal leachate showed the dual importance of NO_3-N and organic C. The largest increase in denitrification was observed when concentration of both NO_3-N and DOC were greatest. Significant correlations between NO_3-N flux into the sediment and denitrification suggested that the water column was supplying NO_3-N for denitrification. While the lack of a significant relationship between denitrification and NO_3-N concentration suggests that concentrations may not have been different enough among treatments to detect this influence. Ammonium and DON concentrations also increased during the leaching process, and as expected did have a significant impact on denitrification rates.

As decomposition and photodegradation caused N to increase in the algal leachate as described above, concurrent increases in dissolved and particulate carbon were also observed. POC increased rapidly, more than tripling in the first 17 hours of leaching. During this same period, DOC concentrations increased to 28% greater than unamended stream water. While the quantity of carbon did not change appreciably, the quality of the organic material was dramatically different. Elevated DOC concentrations due to decomposition of the algal biomass resulted in a significant increase in the fraction of labile carbon available to both aerobic heterotrophs and denitrifiers.

In wetland and stream ecosystems, the quality and quantity of organic matter are important controls on denitrification, especially in systems with high NO_3-N availability (Bachand 2000, Sobczak et al. 2002, Sirivedhin and Gray 2006). Schipper et al. (1994) found that denitrification rates were five times greater upon addition of watercress and fresh pine needles to riparian soils compared to the addition of senescent pine needles. Since the same amount of C was added in each treatment, the researchers concluded that C lability was of greater importance than the quantity of C. In an upland stream in New York, USA, river water was amended with leaf leachate in a series of mesocosms that simulated hyporheic flowpaths (Sobczak et al. 2002). Marked declines in DOC and complete removal of NO_3-N (attributed to denitrification and bacterial assimilation) were observed in the amended mesocosms.

In this study, higher denitrification rates after addition of the algal leachate may have also depended on the quality of C. Lower ratios of C:N in the algal biomass and the dissolved C:N in the leachate after decomposition were correlated with the highest denitrification rates. In a study in wetland sediment, C:N ratios of plant detritus were used as an estimate of the quality of organic material and were similarly correlated with denitrification rates (Bastviken et al. 2005).

Biodegradability of DOC was estimated in water samples by comparing O_2 consumption rates among the controls and treatments amended with algal leachate. Dissolved organic C concentrations were significantly correlated with O_2 flux in the water blanks indicating that the additional C supplied by the algal leachate was a more labile fraction than that in the ambient stream water. The dependence of denitrification on organic C quality was demonstrated in significant linear relationships between denitrification and water column O_2 flux and C:N ratios. These measures of C quality supported the hypothesis that the algal C source was more bioavailable and that it became more labile during the leaching process due to bacterial decomposition. Additionally, these results illustrate the dual dependence of denitrification on C quality and NO_3-N supply.

4.5 Conclusions

Assimilation of watershed derived N by algae lead to large blooms of floating algal mats in these agricultural streams. As this source of organic material decomposed, dissolved organic N and C as well as remineralized inorganic N were released back to the stream. The hypothesis was that this regenerated N would provide an important source of NO_3-N and labile C to denitrifiers and stimulate denitrification rates. Comparisons of the additional N and C supplied by senescing algae had a great impact on water column concentrations. There was a general trend of increased inorganic and organic N concentrations. In addition, measures of the bioavailability of the organic material increased in the amended cores compared to the control and resulted in stimulation of denitrification. Since the additional N in the amended cores was from algal decomposition and algal growth was fueled by nutrients from the watershed, denitrification can be considered a significant removal mechanism for this N source. During the 3-hour experiment, up to 10% of the TN and 100% of the NO_3-N was denitrified. This study demonstrated an important link between the fate of algal-assimilated N and denitrification. If this pattern generally applies to other coastal streams, it has the potential to significantly affect coastal biogeochemistry.

CHAPTER 5:

RESEARCH FINDINGS AND IMPLICATIONS FOR WATERSHED MANAGEMENT

5.1 Summary of research findings

5.1.1 Linking hydrology and biogeochemistry

<u>Objective</u>: Elucidate the linkage between hydrology in modified stream networks and nutrient retention in coastal watersheds in close proximity to sensitive estuarine waters

<u>Conclusions</u>: This study illustrated the importance of including both short term (storm events) and long term (seasonal) temporal influences when placing nutrient retention in the context of the larger stream network. Storm events played a disproportionately large role in export of nitrate (NO_3-N) and phosphate (PO_4-P) from the agricultural watershed, while seasonal changes in biological activity increased ammonium (NH_4-N) retention during the warm spring months following fertilization. Antecedent conditions were shown to significantly affect NO_3-N export, with higher storm event-averaged concentrations following prolonged dry periods compared to wet periods. These results are similar to those observed in other agricultural watersheds (Petry et al. 2002, Poor and McDonnell 2007) but has not yet been described in coastal agricultural catchments with relatively flat topography.

Although actively managed for tree production, the silvicultural watershed displayed nutrient and C cycling characteristics similar to other natural forested watersheds (Boyer et al. 1997, Creed and Band 1998, Inamdar et al. 2004). In fact, the mean total dissolved nitrogen (N) load was approximately 0.95 kg ha^{-1} y^{-1}, which was 50% lower than the mean value (1.97 kg ha^{-1} y^{-1}) reported for minimally disturbed watersheds throughout the U.S. (Lewis Jr. 2002). Dissolved organic carbon (DOC) dynamics were also similar to natural forests. Concentrations of DOC were strongly influenced by precipitation as terrestrial storage zones were flushed to the stream during storm events.

Results from the drainage network model and the reach-scale mass balance showed significant instream retention of NH_4-N and PO_4-P. Seasonal variations were also observed highlighting the importance of temporal changes such as climate fluctuations (temperature and precipitation) and crop management activities (fertilizer application during the spring, rapid plant growth during the summer and fall harvest).

5.1.2 Spatial and temporal variability of denitrification

Objective: Characterize spatial and temporal patterns of denitrification in headwater stream sediments and quantify the potential for denitrification to remove NO_3-N from the stream network.

Conclusions: Denitrification rates measured in the streambed sediments in both agricultural and silvicultural watersheds were highest in the 1st order streams and decreased as stream order decreased. During baseflow conditions, these drainage systems function like wetlands with intensive recycling of nutrients and C among closely connected assemblages of vascular plants, attached microalgae and bacteria. During these times, denitrification was potentially coupled with nitrification due low instream NO_3-N concentrations and slow diffusion of streamwater NO_3-N to sediment communities (Seitzinger 1994, Cornwell et al. 1999, Kemp and Dodds 2002a). Temporal variability was also observed with highest denitrification rates occurring during the spring compared to other seasons (Christensen et al. 1990, Pattinson et al. 1998, Pinay et al. 2000). This spring maximum in biological activity was likely to due to increased temperature and fertilizer application in the agricultural watershed.

5.1.3 Variables affecting denitrification

Objective: Identify the factors controlling denitrification rates in headwater streams in two contrasting land uses: agricultural and silvicultural.

Conclusions: Changes in hydrology accompanying land use changes are particularly critical in predicting N delivery to sensitive estuarine systems downstream. Land use alone greatly impacted export of dissolved inorganic N (DIN) loads but had a smaller influence on rates of denitrification. Total dissolved N export was significantly greater from the agricultural watershed compared to the silvicultural watershed. Average denitrification rates were greater in the agricultural stream likely due to the combined affect of more readily available organic C sources (i.e. senescing algal biomass) and elevated NO_3-N concentrations (Schipper et al. 1994, Sobczak et al. 2002, Royer et al. 2004, Smith et al. 2006).

In the agricultural stream, denitrification was most strongly controlled by NO_3-N supply with nitrification potentially playing an important role in N cycling during baseflow conditions when instream concentrations were consistently low. Secondarily, rates were influenced by temperature and organic C supply. The importance of quality over quantity of the C source was illustrated by the stimulation of denitrification rates upon addition of a natural labile C source in the form of algal leachate.

5.1.4 Impact of denitrification on nitrate export from the agricultural watershed

Objective: Quantify the contribution of NO_3-N removed in agricultural streambed sediments via denitrification as a proportion of the total instream removal determined from a mass balance analysis.

Conclusions: A mass balance analysis of nutrient retention in a typical 2nd order stream in the agricultural watershed revealed that the stream was efficient at retaining NH_4-N and PO_4-P, but not NO_3-N. In fact, the stream appeared to be gaining NO_3-N along the reach, which points to potential missing and/or under-estimated inputs in the mass balance.

Although denitrification rates were on the lower end of the range of rates measured in other agricultural streams, NO_3-N concentrations were also significantly lower (Christensen et al. 1990, Jansson et al. 1994, Garcia-Ruiz et al. 1998a). When NO_3-N concentrations were raised during enrichment experiments to levels typically observed during storm events, denitrification rates increased proportionally. This indicates a strong potential for denitrification to remove instream NO_3-N. However, in order for denitrification to effectively remove NO_3-N from the water column, hydrodynamic mechanisms must exist to move this nitrate-rich streamwater to sediment communities where denitrification occurs.

Instream nutrient retention by algal assimilation represents a temporary storage of watershed derived N as DIN is converted to algal biomass. As this biomass senesces, DIN and dissolved organic N (DON) is released back to the stream ecosystem and potentially delivered downstream. This concept of downstream movement of N as it cycles between organic N in biomass and DIN in the water column has been described as nutrient spiraling (Newbold et al. 1981). To determine the potential for this remineralized N to be denitrified, experiments were conducted comparing denitrification rates in sediments amended with leachate derived from senescing algal biomass. Denitrification rates were significantly greater in sediments amended with algal leachate compared to ambient streamwater. Estimates of the proportion of remineralized N that was denitrified during these experiments showed that the potential for denitrification to remove this "re-released" N is significant in these streams.

5.2 Agricultural management implications

In the agricultural stream network in this study, denitrification was most strongly influenced by NO_3-N supply. During storm events, pulses of NO_3-N were observed, often increasing instream concentrations by 1-2 orders of magnitude. Experimental enrichments showed the potential for denitrification rates to increase in direct proportion to this increased NO_3-N source, however this NO_3-N -rich streamwater must first be transported to denitrifying communities in these channelized streams. Management of these drainage networks, including channel modifications to increase hyporheic flow (i.e. addition of woody debris or other channel obstructions) or increase retention time (i.e. flashboard risers or streamside wetlands) may help reduce downstream export in streams that support high rates of denitrification.

The results from the NO_3-N mass balance indicated the potential for missing and/or underestimated sources along this 2nd order stream. One likely source is underestimated NO_3-N load from groundwater. During storm events, sustained NO_3-N pulses were often observed indicating the potential for significant terrestrial storage of NO_3-N. In the absence of riparian vegetation, this groundwater flows virtually unimpeded to the streams where high stream flows quickly transport it downstream.

High biological activity and removal of NO_3-N via denitrification has been shown in riparian zones (Peterjohn and Correll 1984, Lowrance et al. 2000). Establishment of riparian buffers along the low order channelized stream could significantly reduce instream N.

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